

# Construction of Pbs.PGK.PCR1

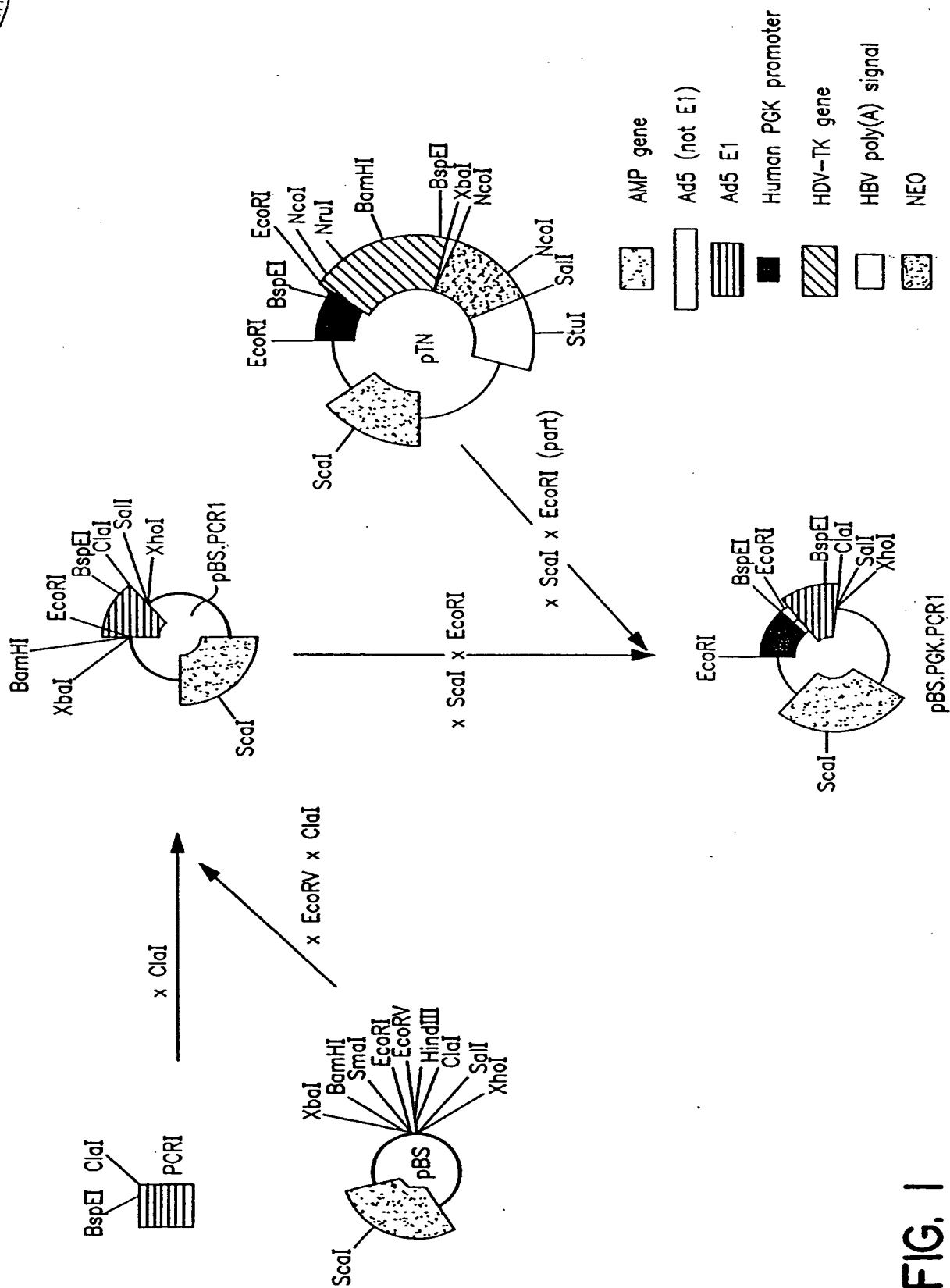


FIG. 1

Construction of pIG.E1a.E1b.X

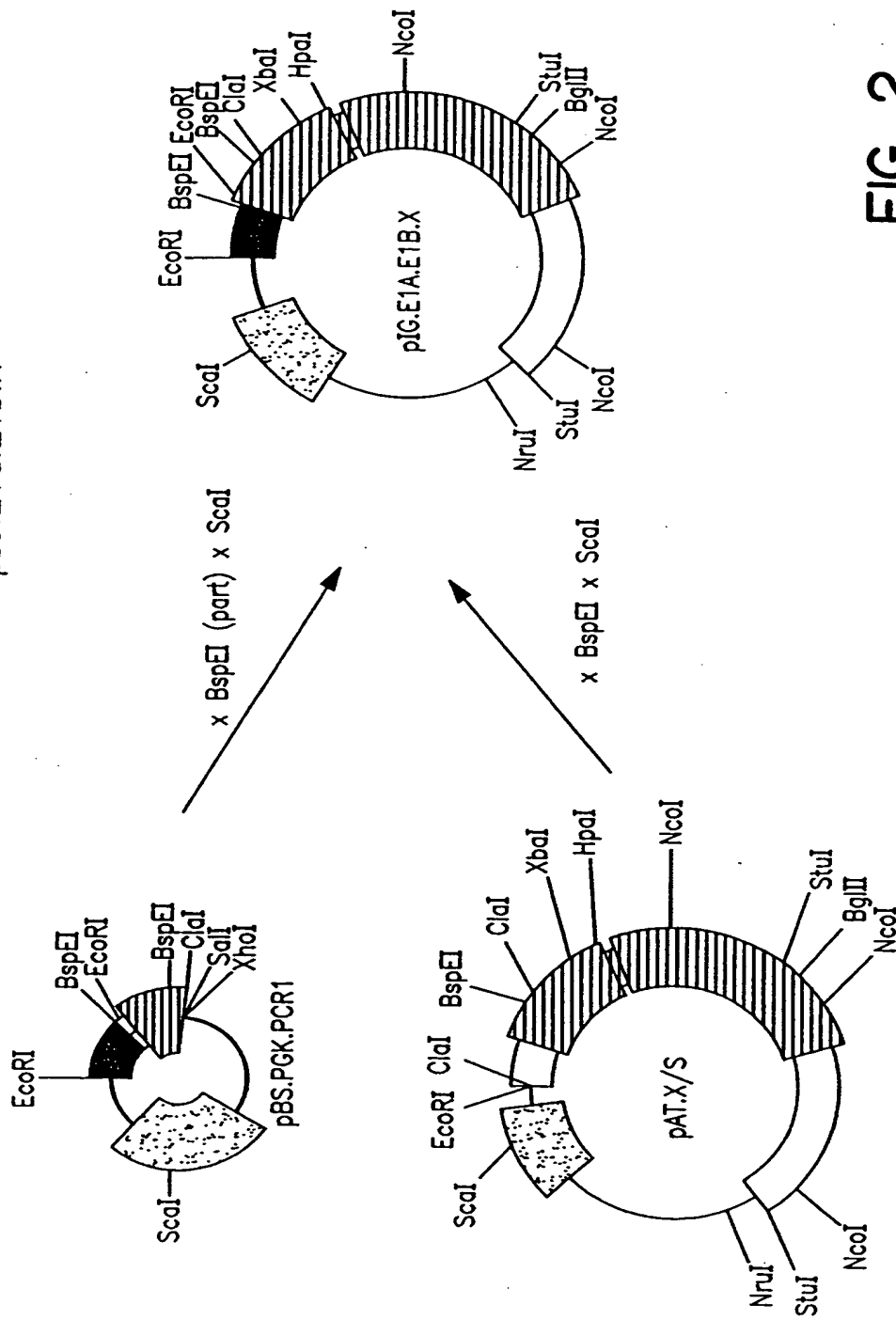


FIG. 2

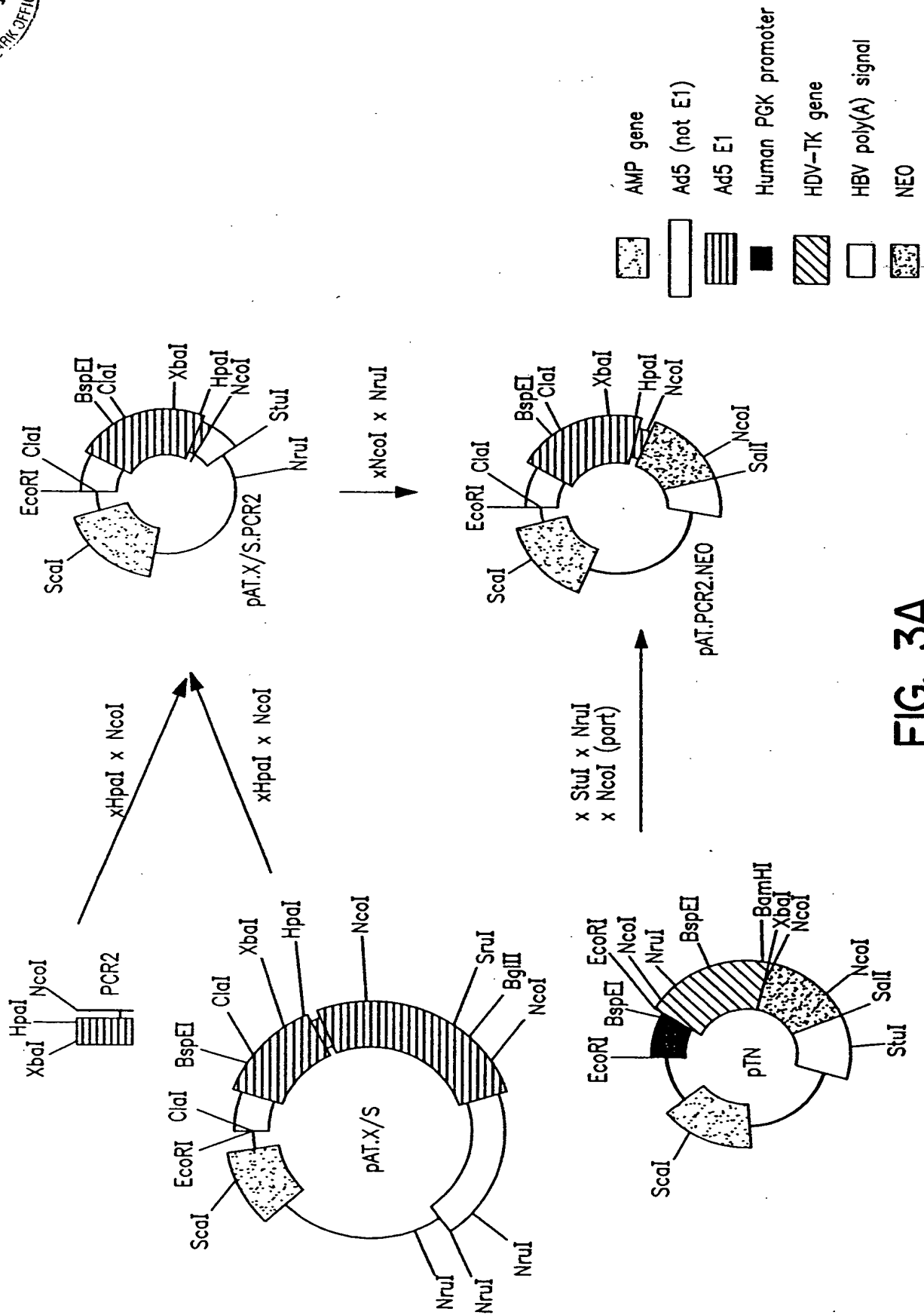


FIG. 3A

# Construction of pIG.E1a.NEO

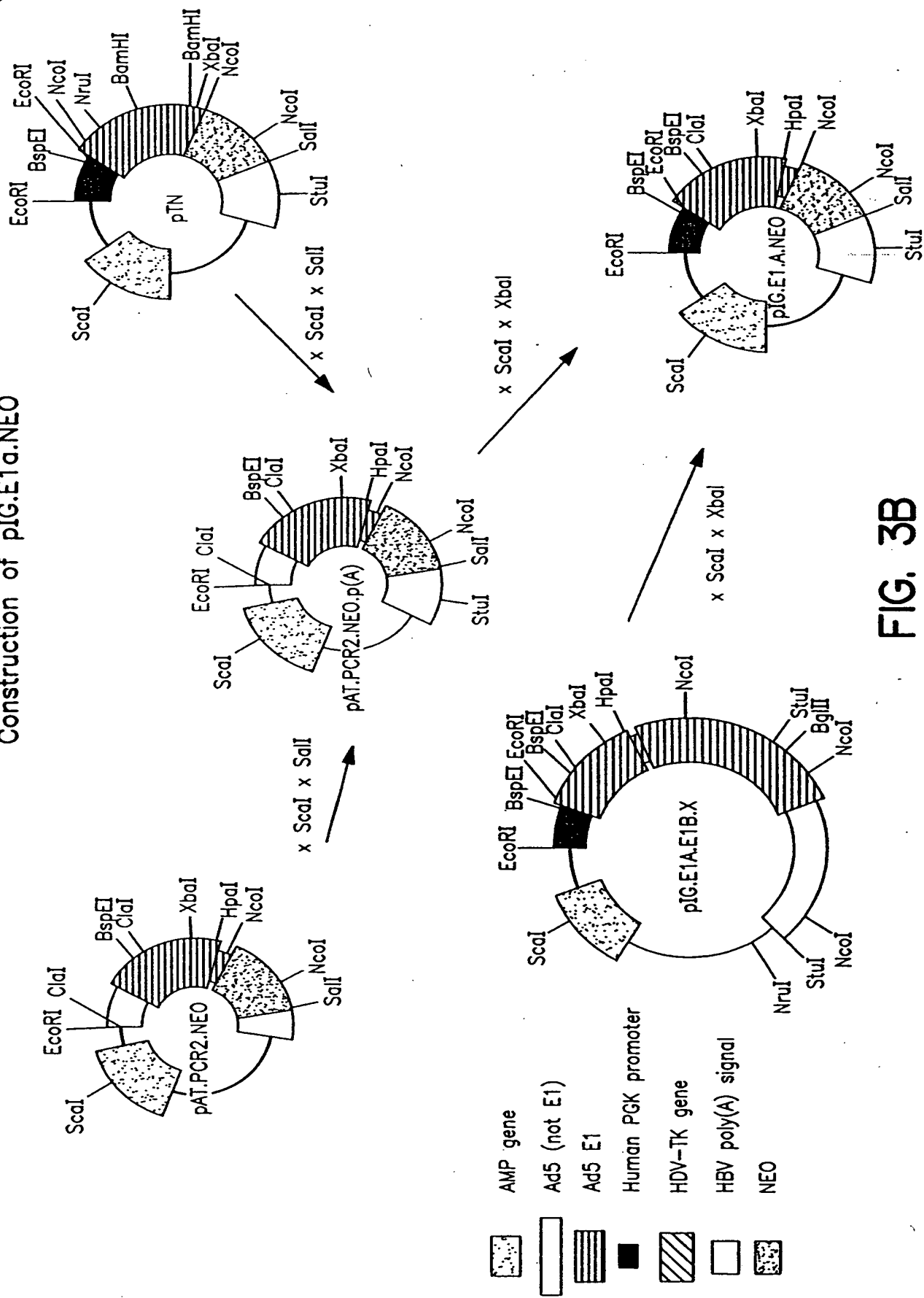
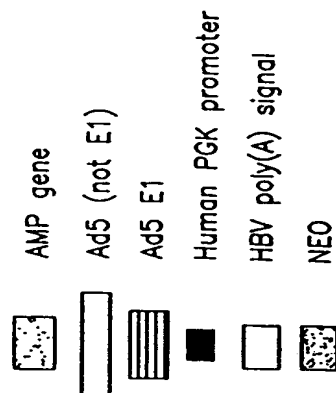
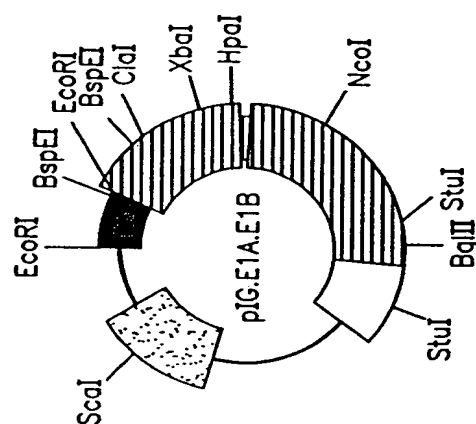
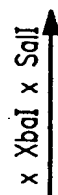
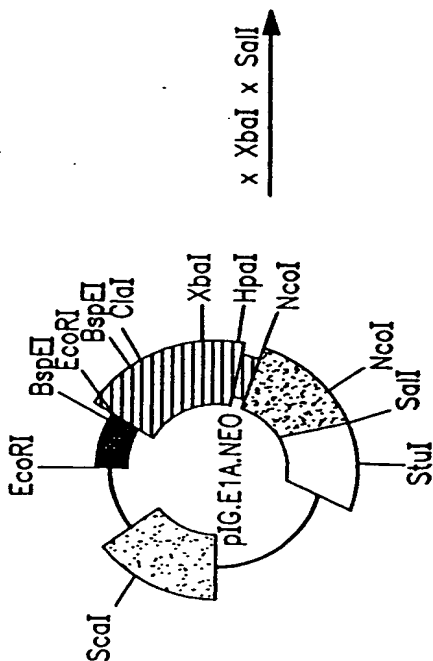
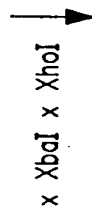
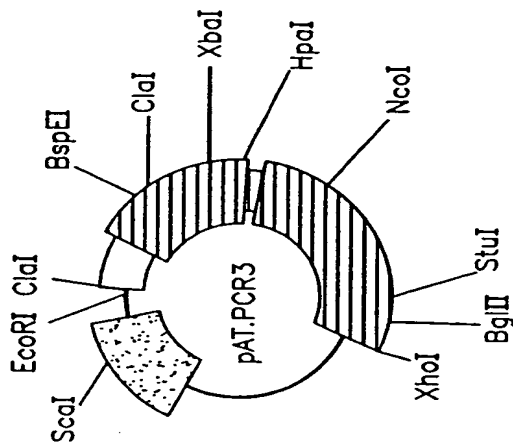
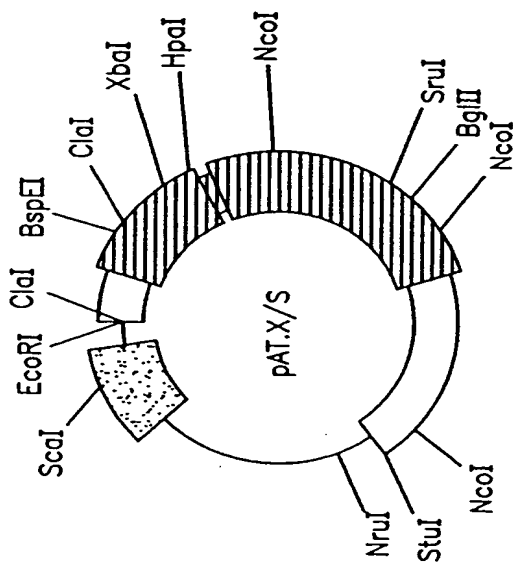
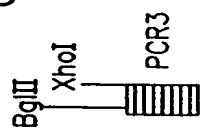


FIG. 3B

FIG. 4

Constitution of pIG.E1a.E1b



# Construction of pIG.NEO

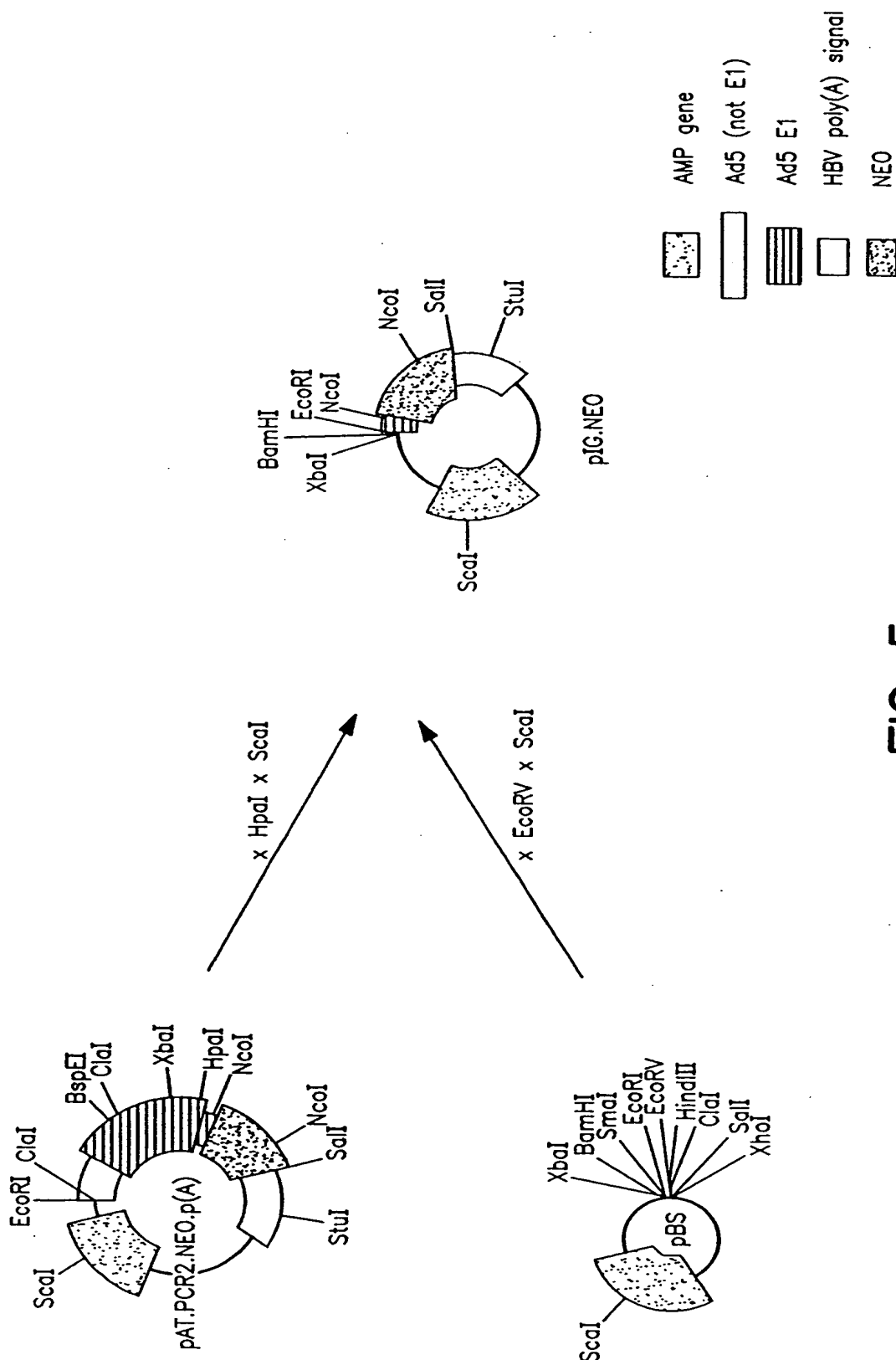
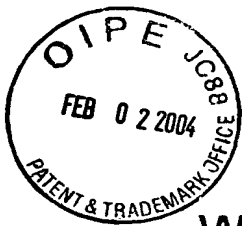


FIG. 5





Western blotting analysis of A549 clones transfected with pIG.E1A.NEO and PER clones (HER cells transfected with pIG.E1A.E1B)

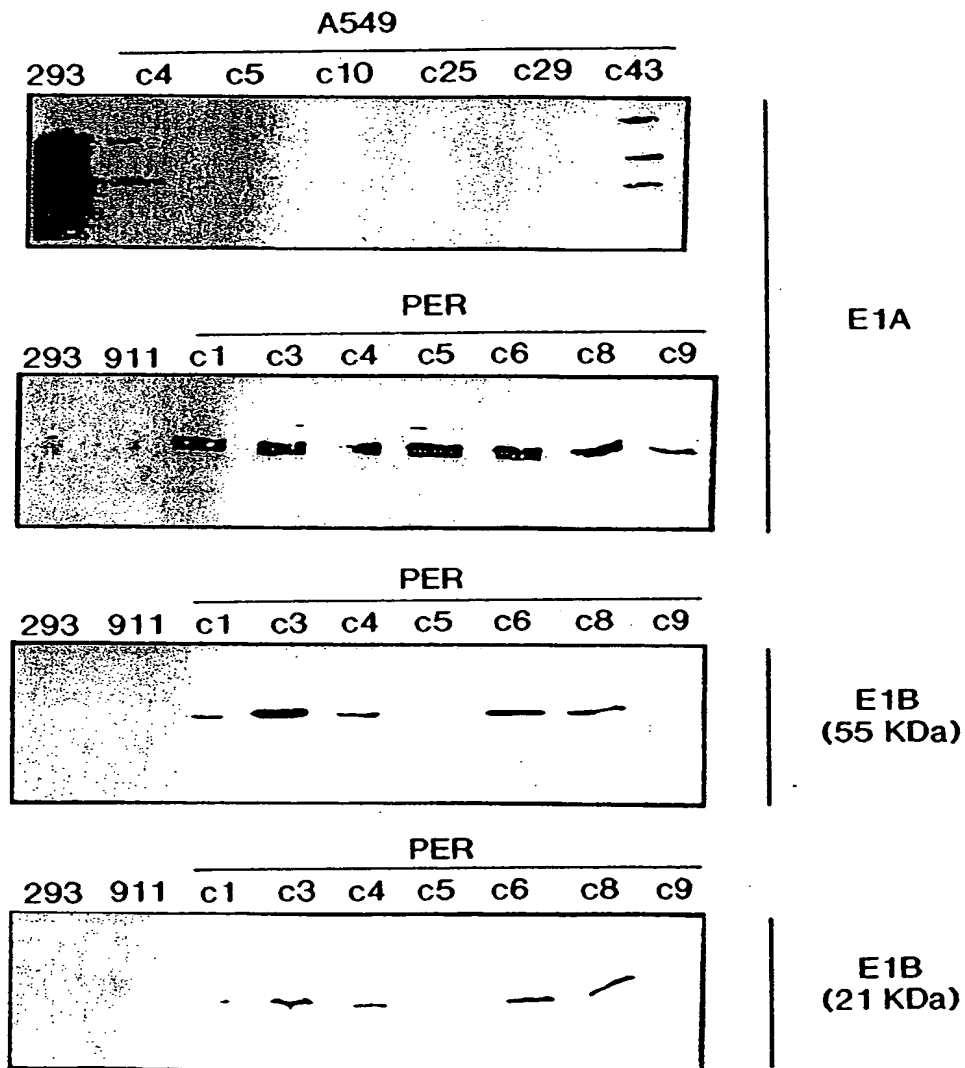


FIG. 7





## Southern blot analyses of 293, 911 and PER cell lines

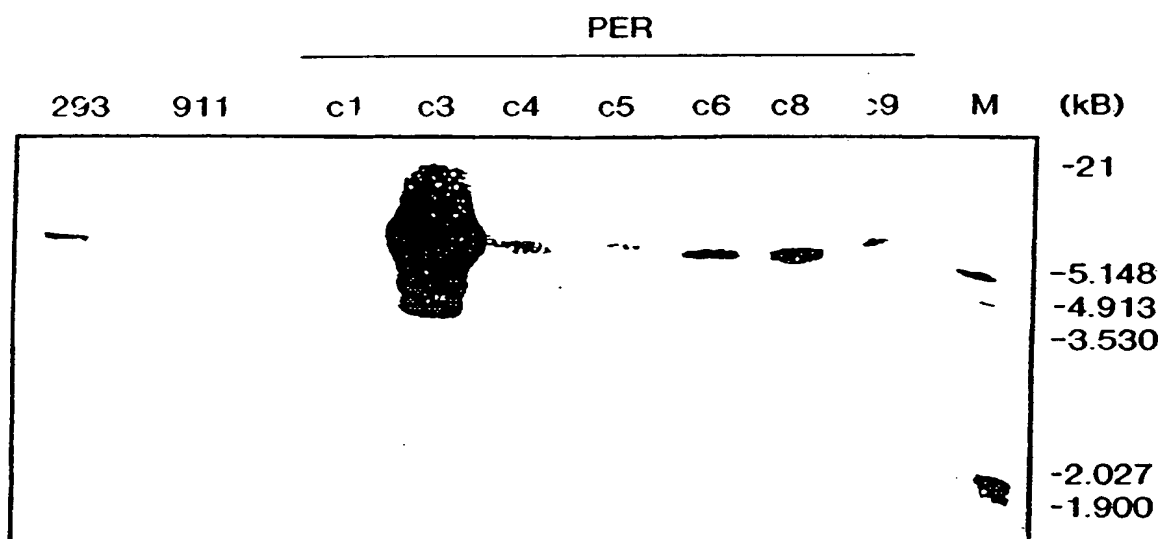


FIG. 8



Transfection efficiency of PER.C3, PER.C5, PER.C6 and 911 cells. Cells were cultured in 6-well plates and transfected (n=2) with 5  $\mu$ g pRSV.lacZ by calcium-phosphate co-precipitation. Forty-eight hours later the cells were stained with X-GAL. The mean percentage of blue cells is shown.

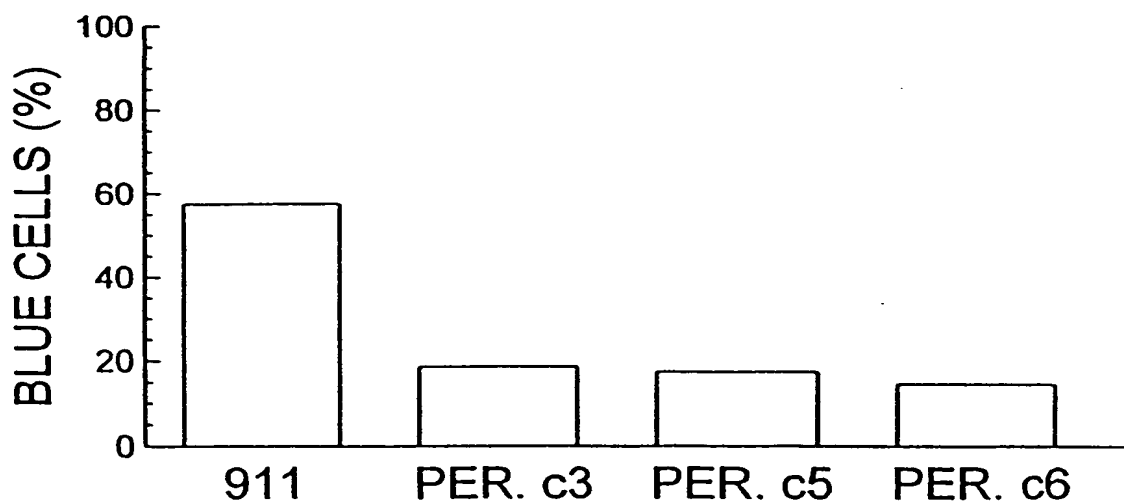
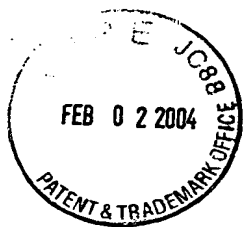


FIG. 9



## Construction of pMLP1.TK

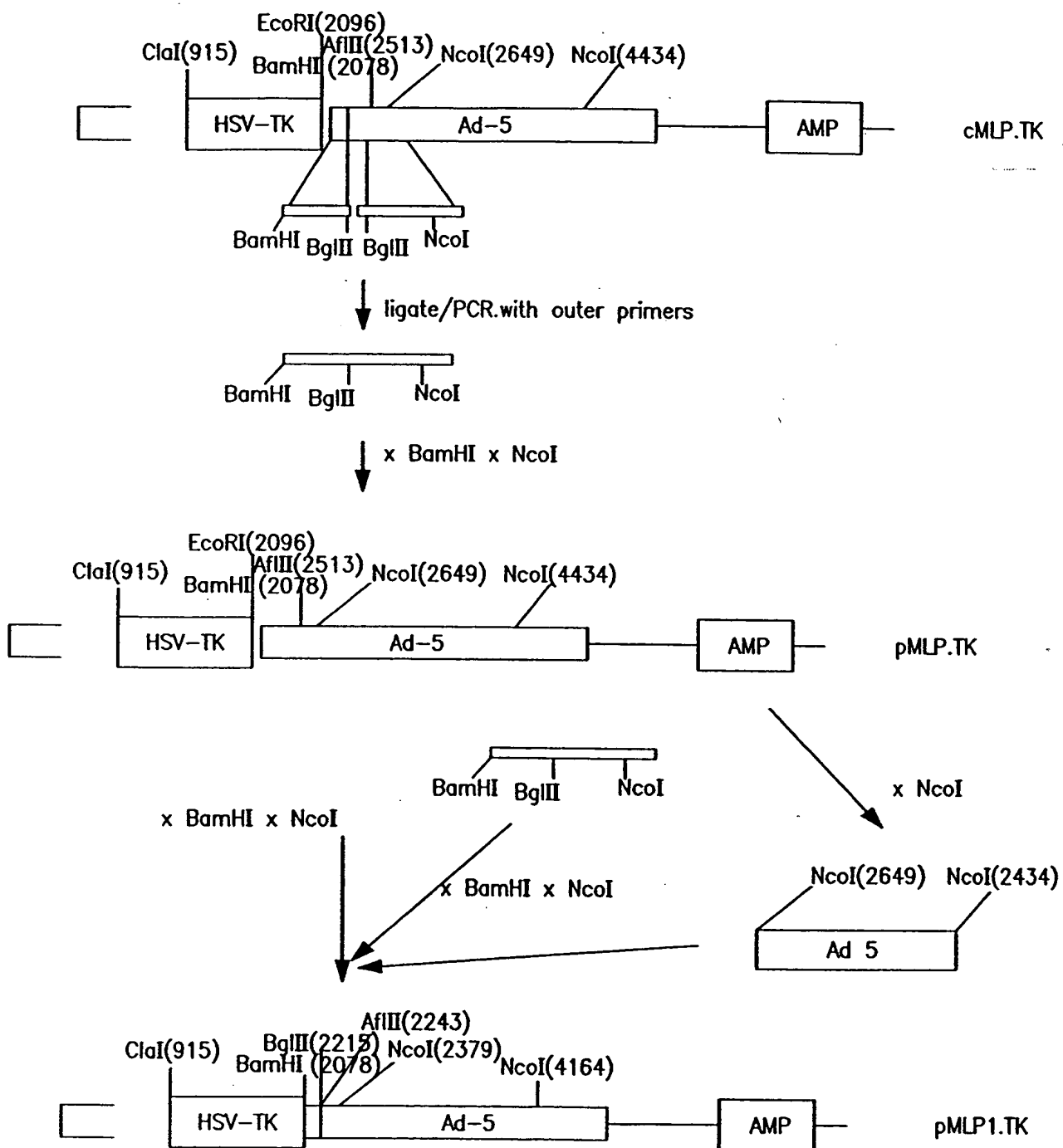


FIG. 10



# New recombinant adenoviruses and packaging constructs without sequence overlap

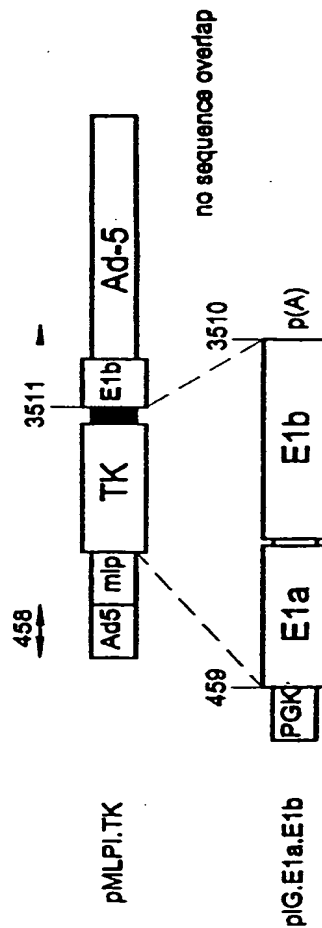
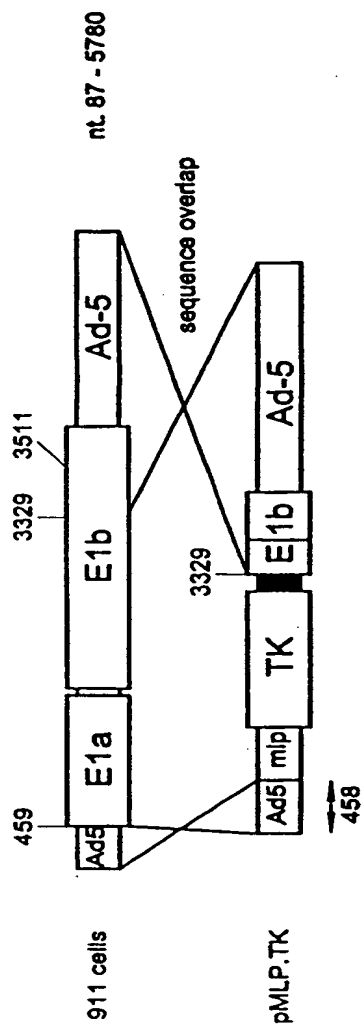
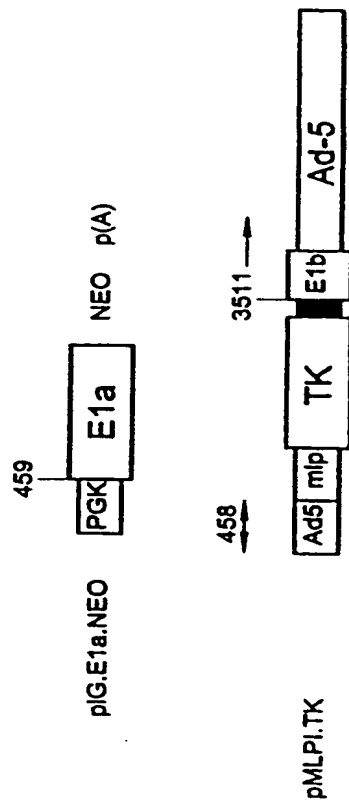


FIG. 1IA

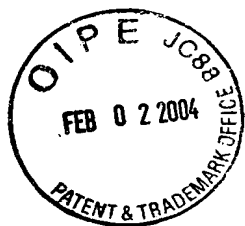
Packaging system based on primary cells



New recombinant adenoviruses and packaging constructs without sequence overlap



Packaging system based on established cell lines: transfection with E1a and selection with G418 **FIG. 1B**



# Generation of recombinant adenovirus

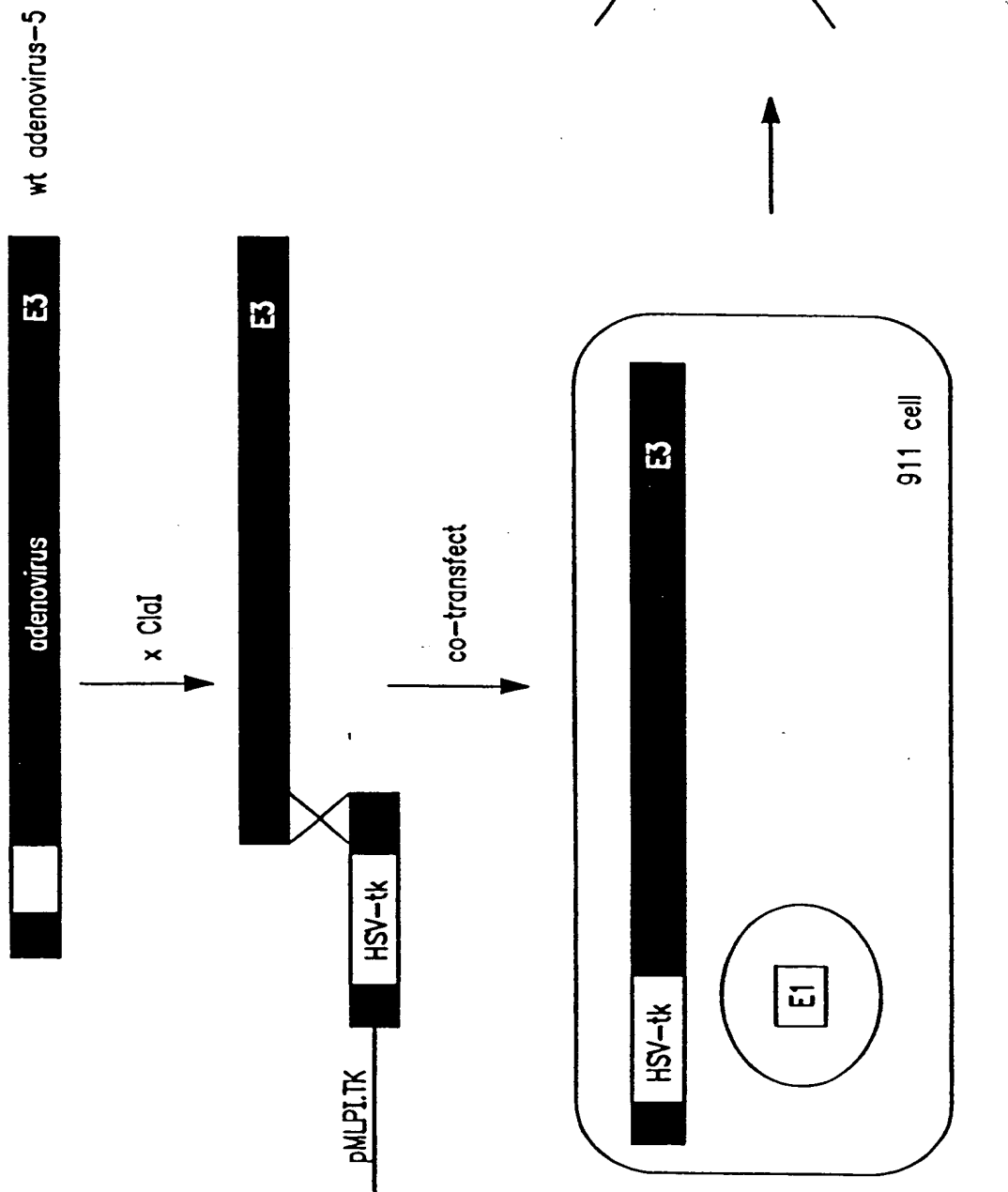


FIG. 12

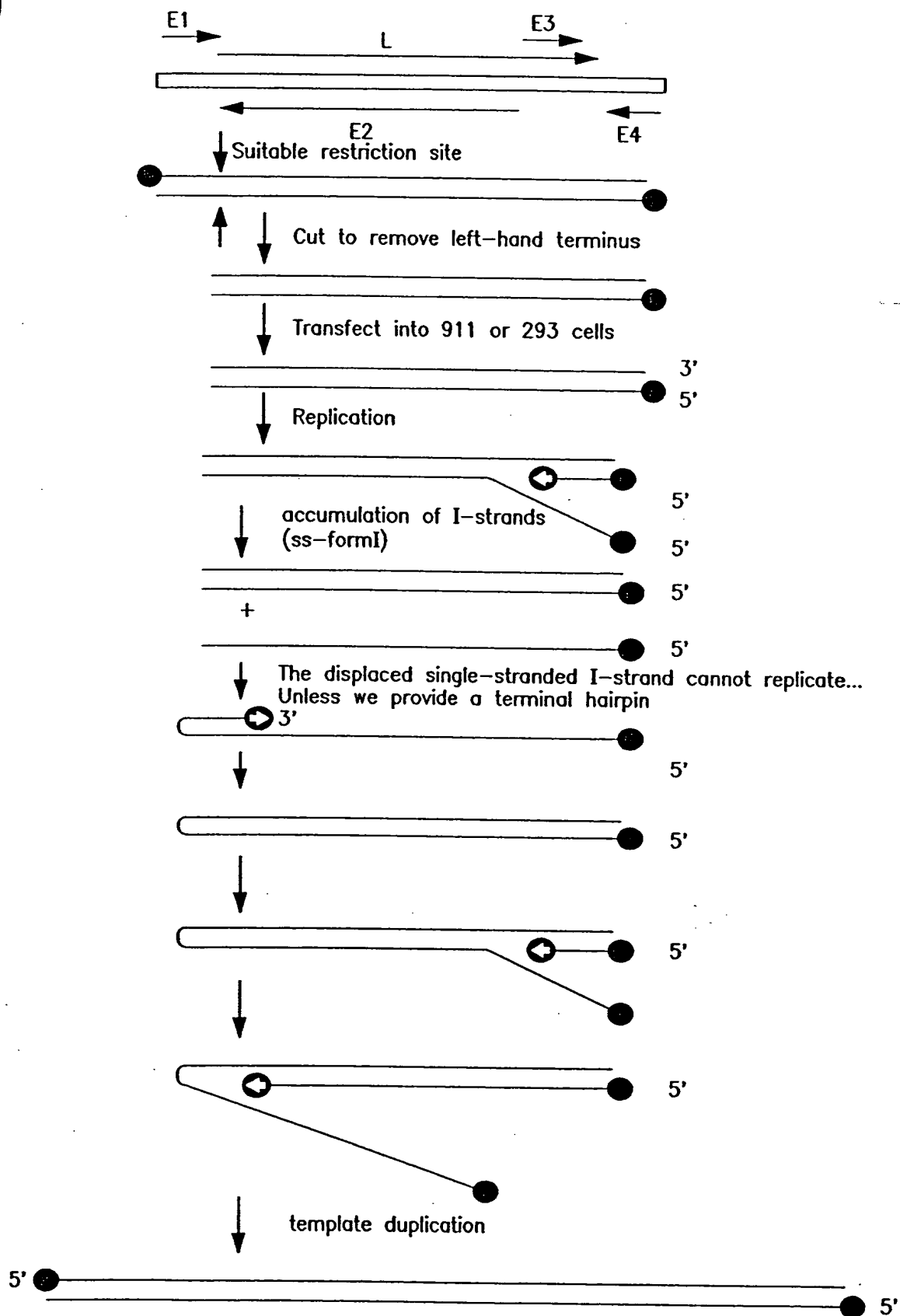
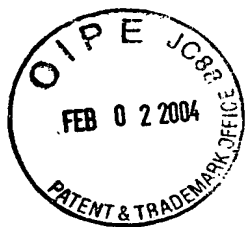
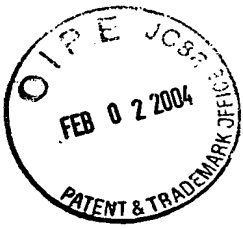


FIG. 13



# Replication of Adenovirus

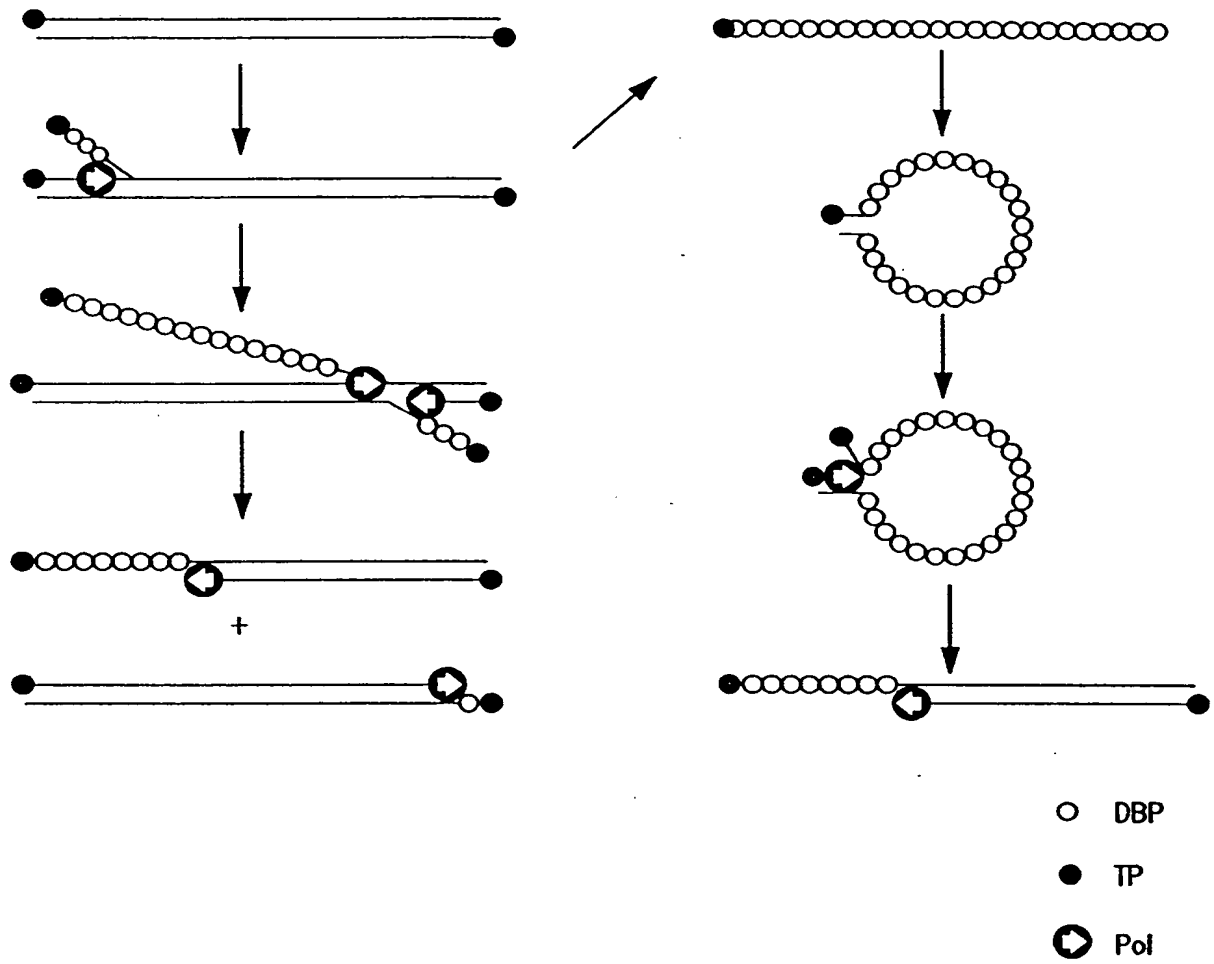
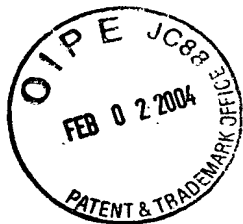


FIG. 14





The potential hairpin conformation of a single-stranded DNA molecule that contains the HP/asp sequences used in these studies. Restriction with the restriction endonucleases *Asp718I* of plasmid pICLHa, containing the annealed oligonucleotide pair HP/asp1 and HP/asp2 will yield a linear double-stranded DNA fragment. In cells in which the required adenovirus genes are present, replication can initiate at the terminus that contains the ITR sequence. During the chain elongation, the one of the strands will be displaced. The terminus of the single-stranded displaced-strand molecule can adopt the conformation depicted above. In this conformation the free 3'-terminus can serve as a primer for the cellular and/or adenovirus DNA polymerase, resulting in conversion of the displaced strand in a double-stranded form.

```
5'-GTACACTGACCTAGTGCCGCCCGGGCA
      ||||| A
3'-GATCACGGCGGGCCCGA
```

FIG. 15

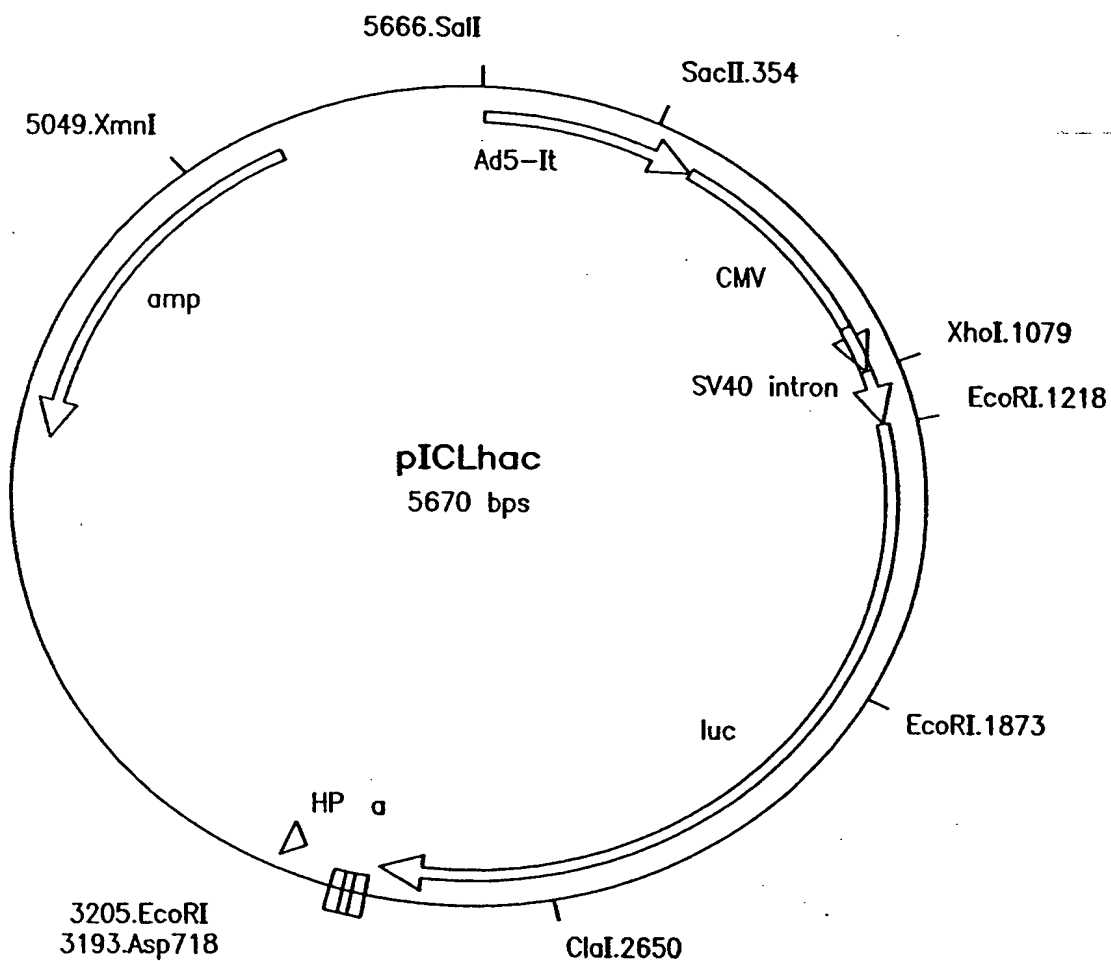
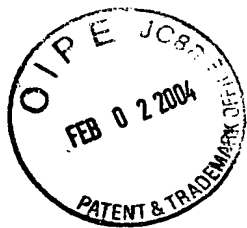


FIG. 16

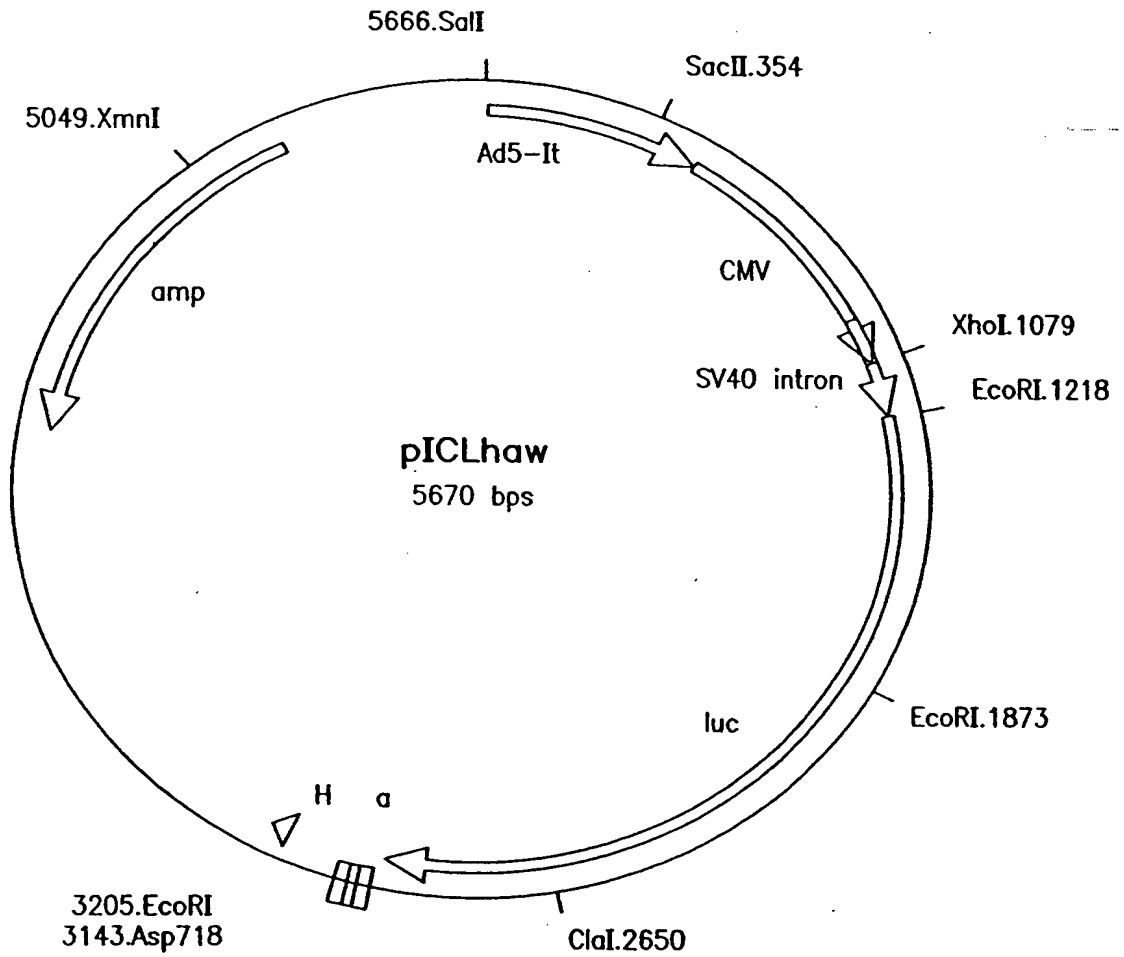
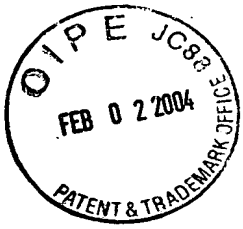


FIG. 17

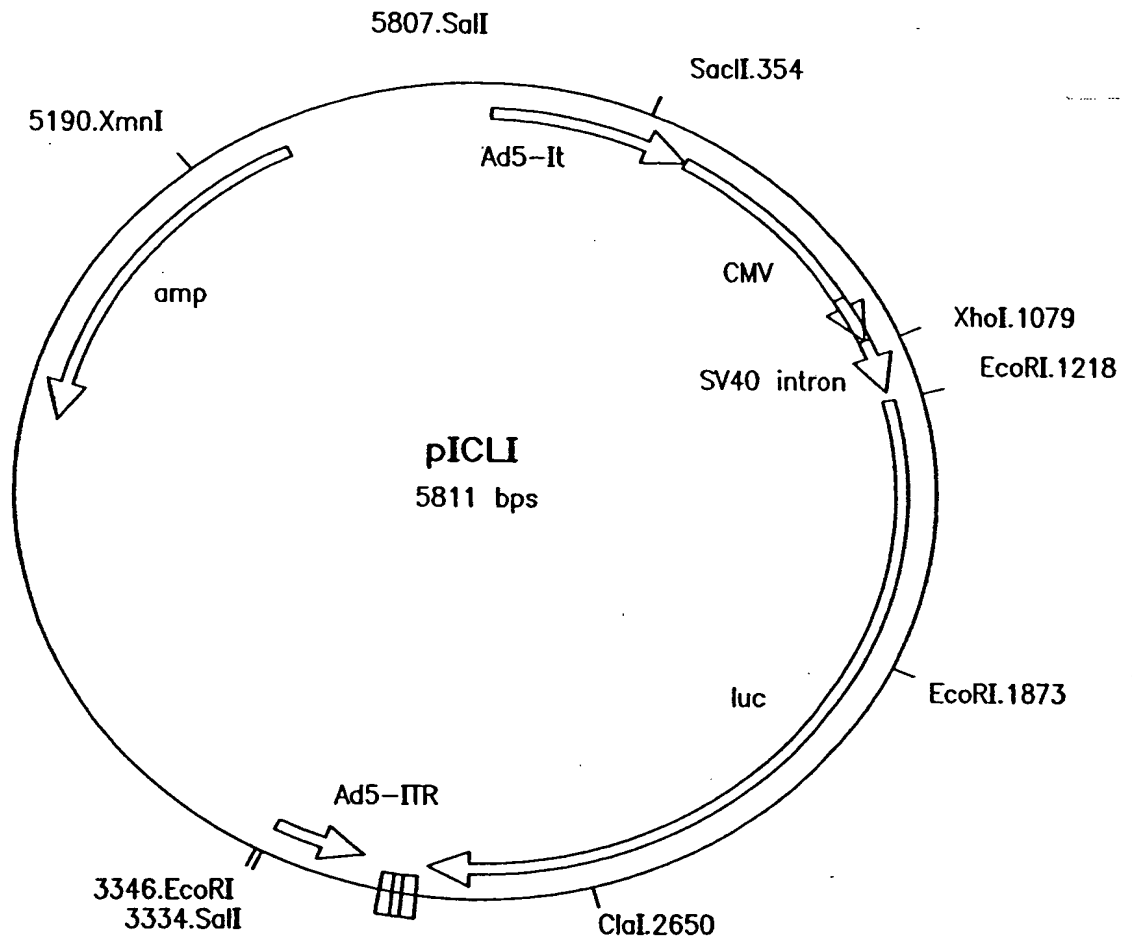
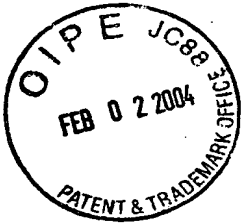


FIG. 18

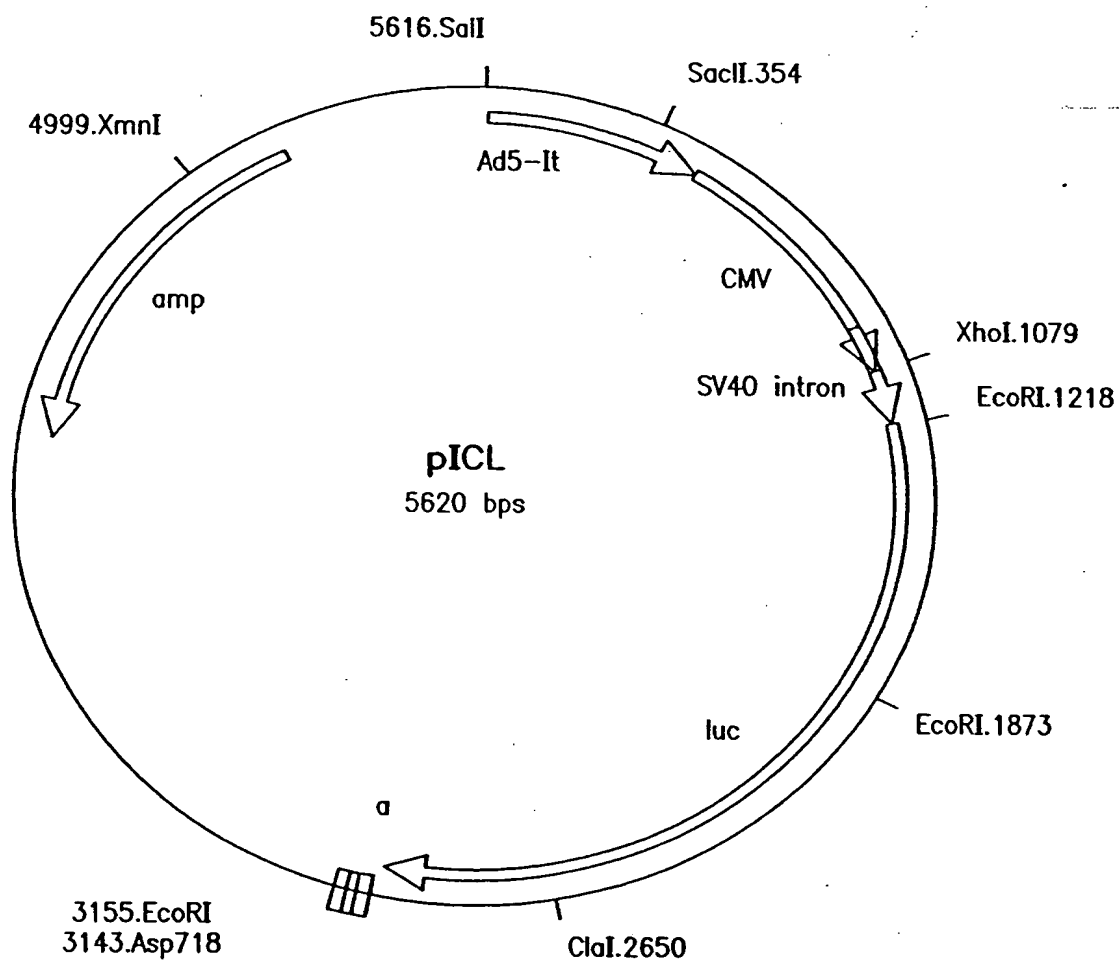
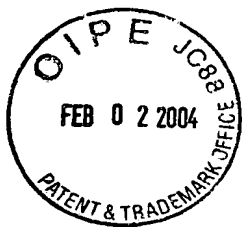


FIG. 19



## Cloned adenovirous fragments

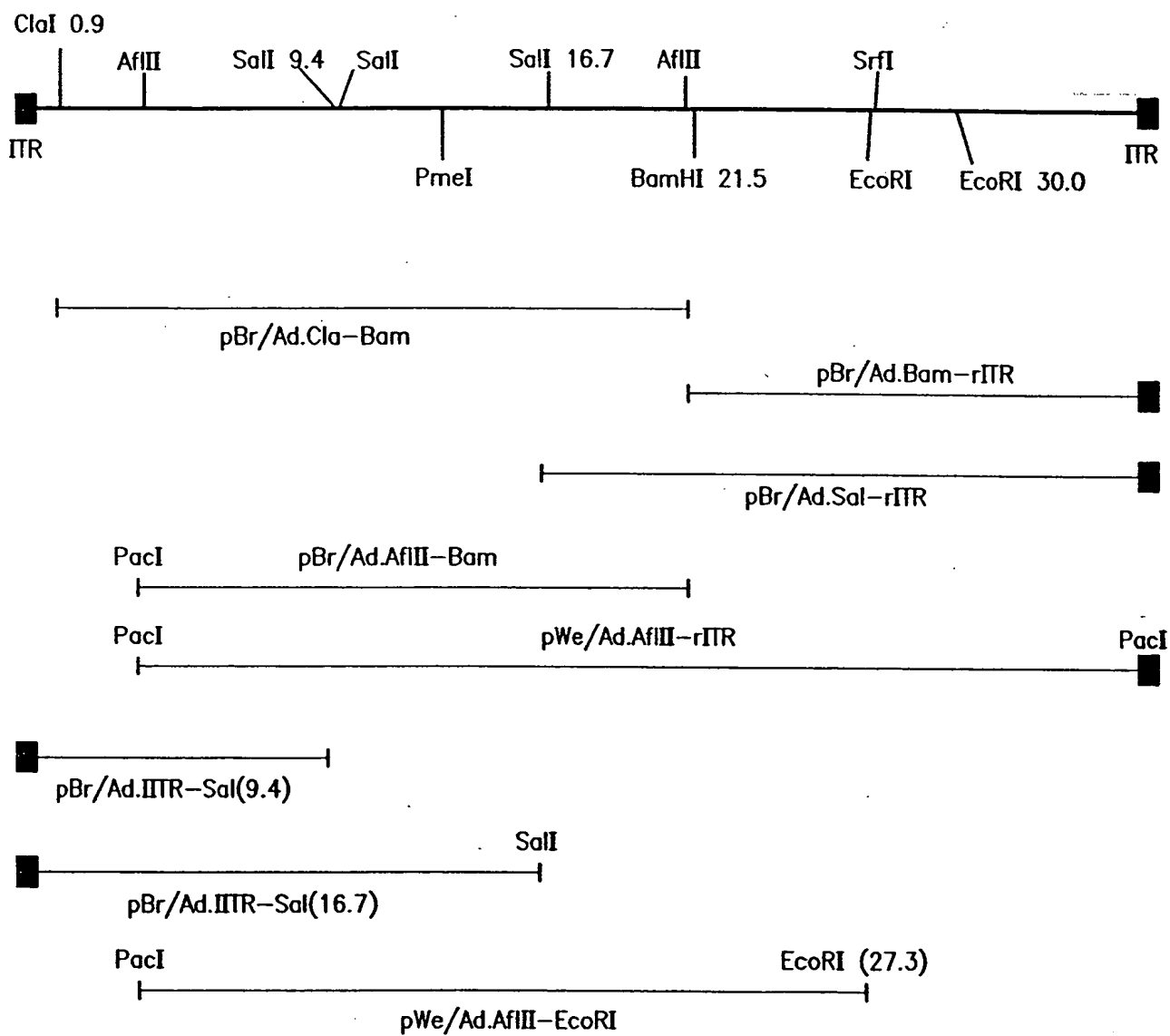


FIG. 20



# Adapter plasmid pAd5/L420-HSA

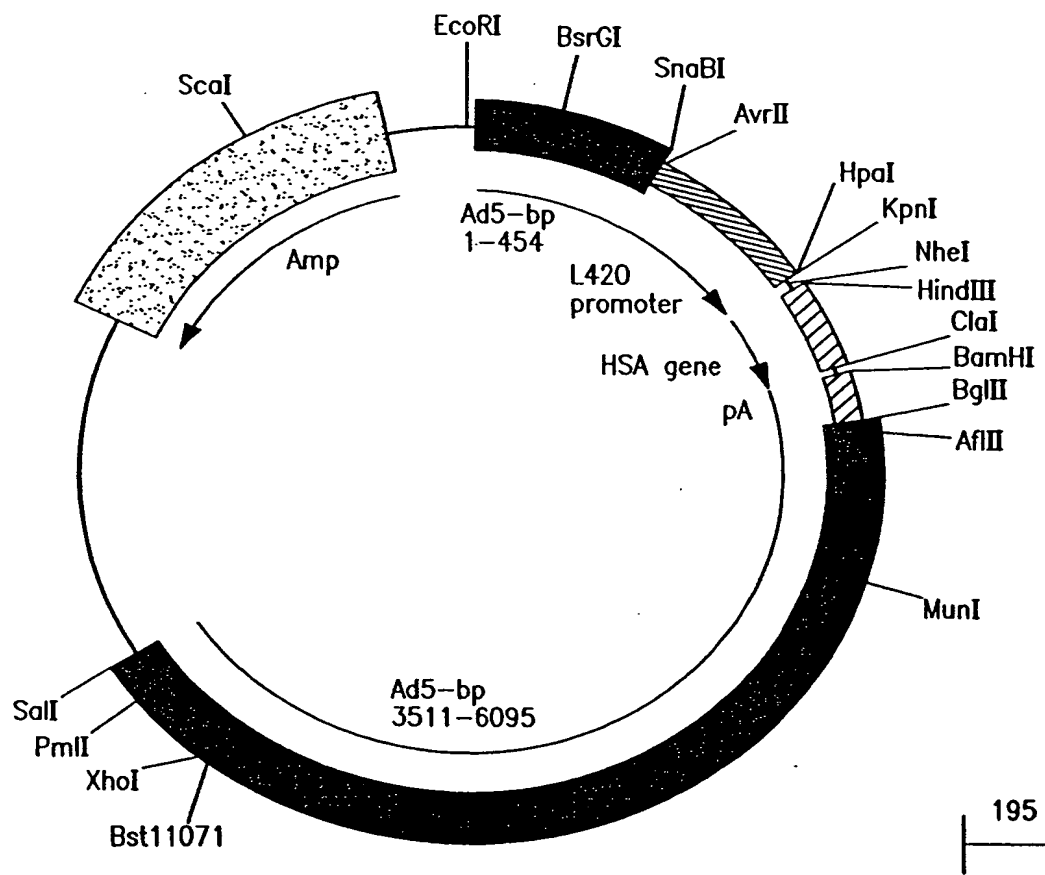


FIG. 21



Adapter plasmid pAd5/CLIP

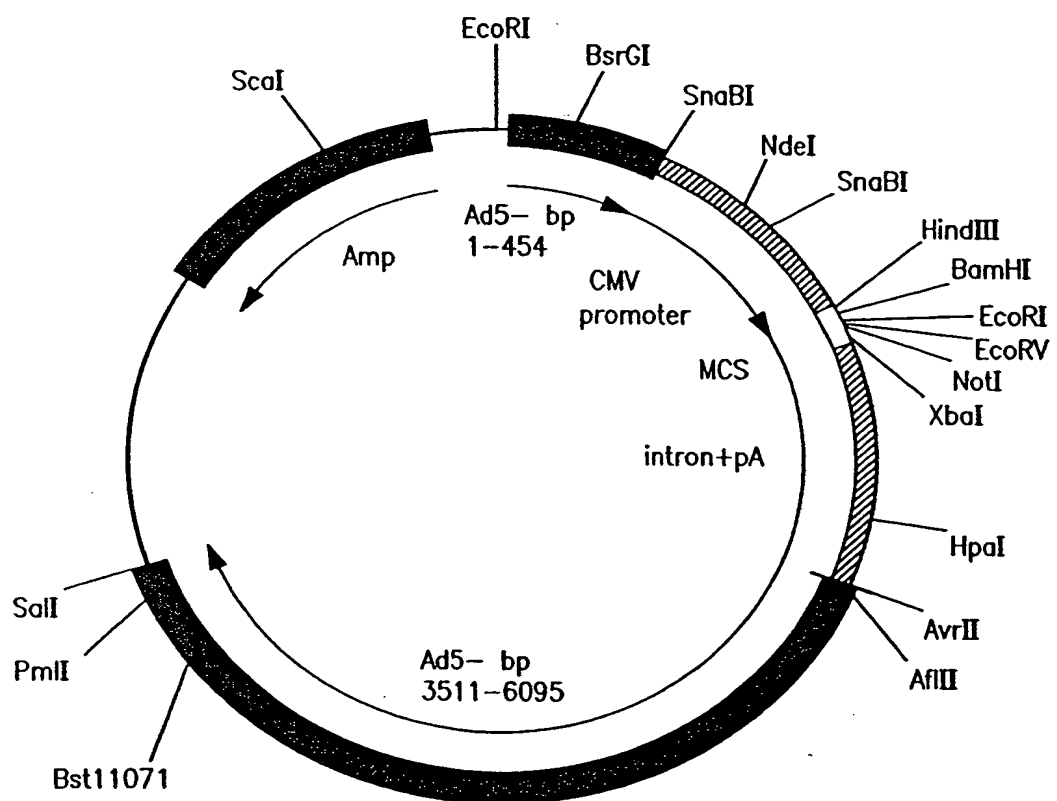


FIG. 22



# Generation of recombinant adenoviruses

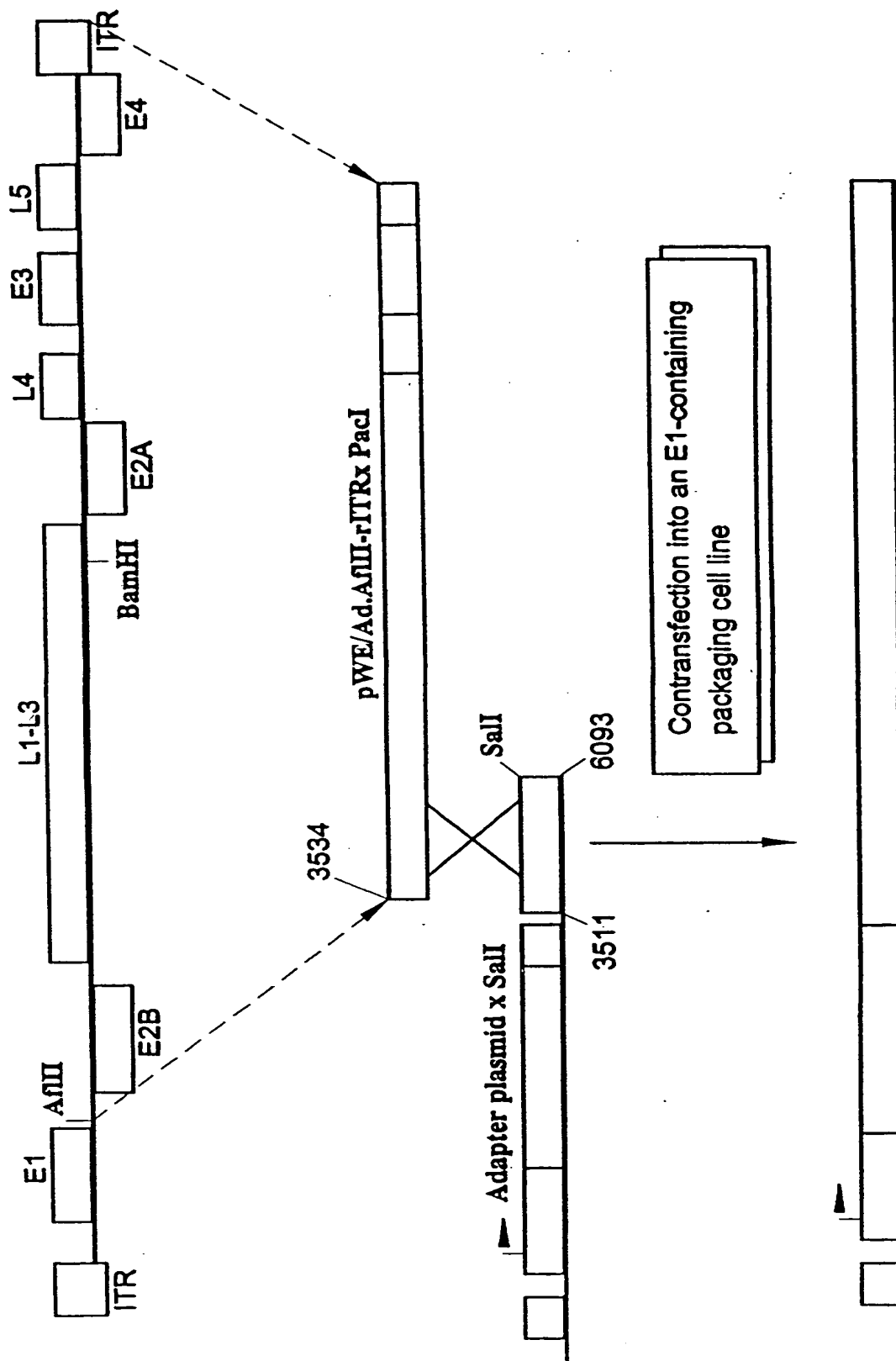


FIG. 23



# Minimal adenovirus vector pMV/L420H

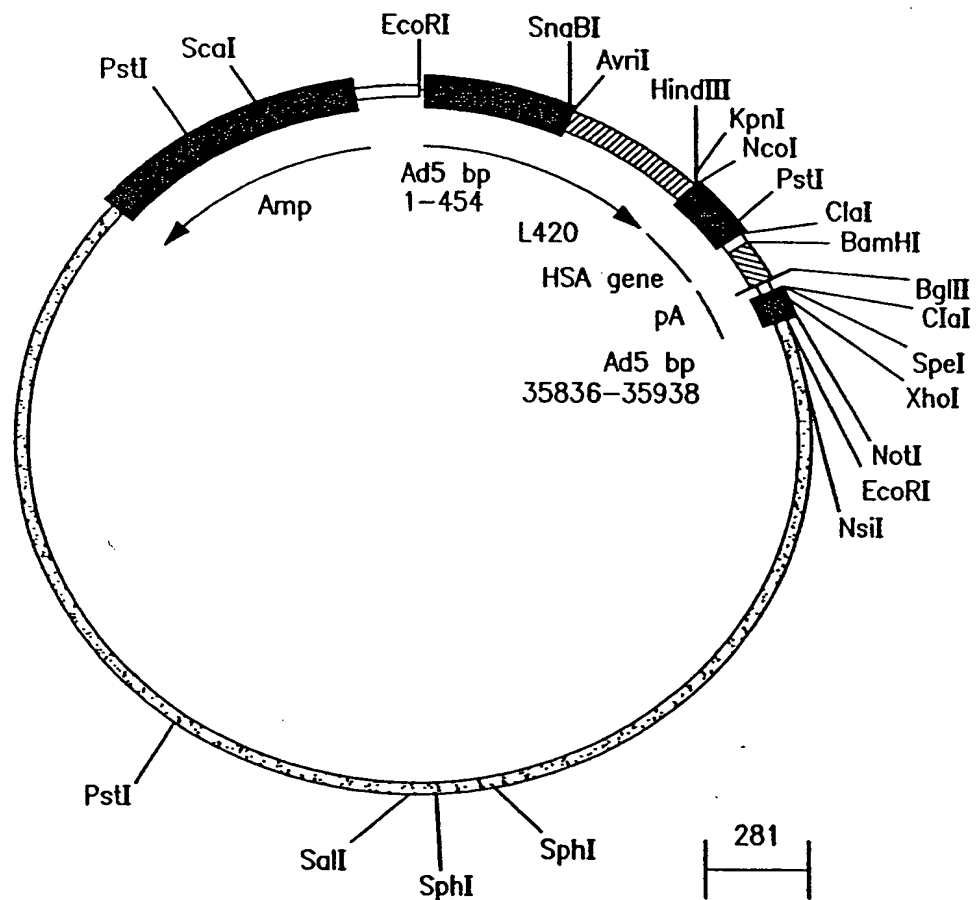
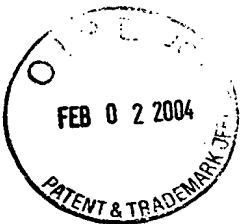


FIG. 24



# Construction of pWE/AdΔ5'

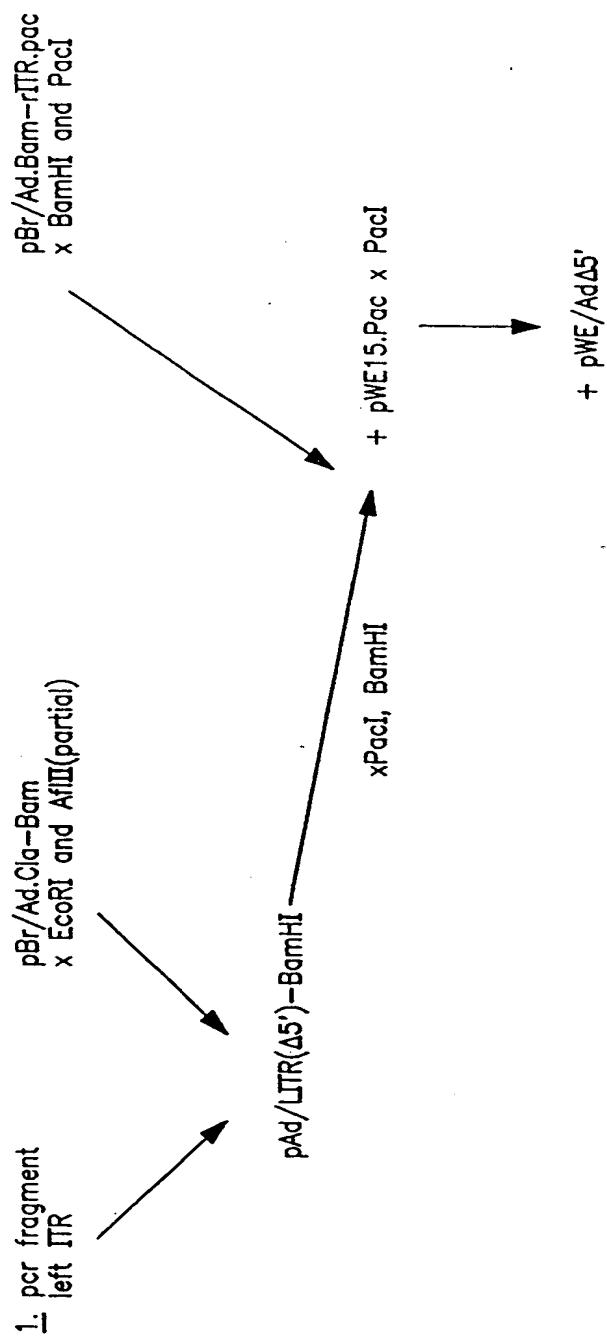
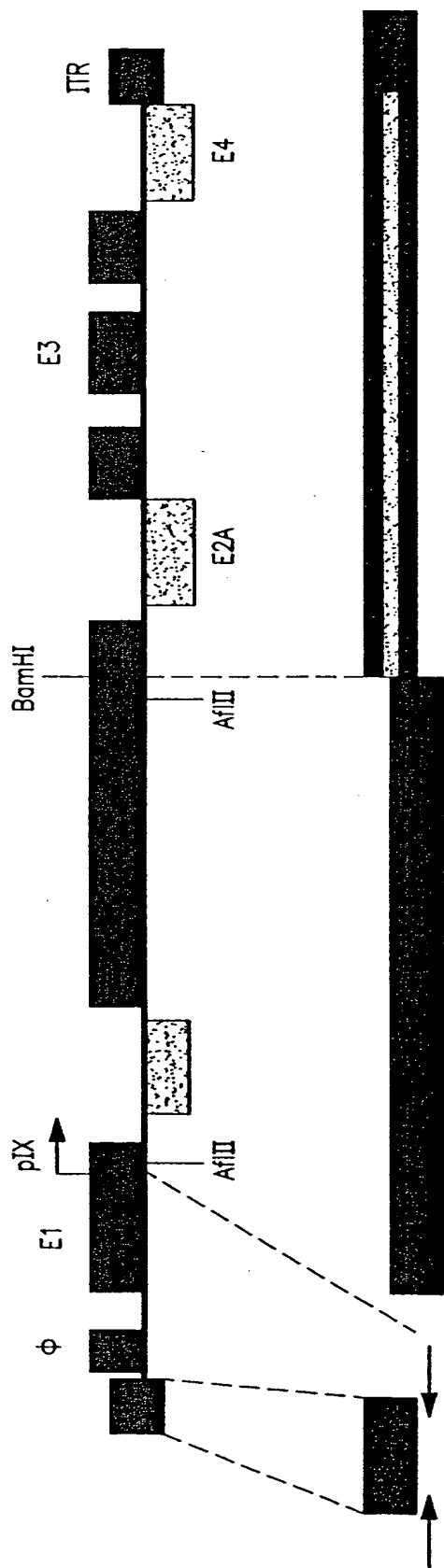


FIG. 25

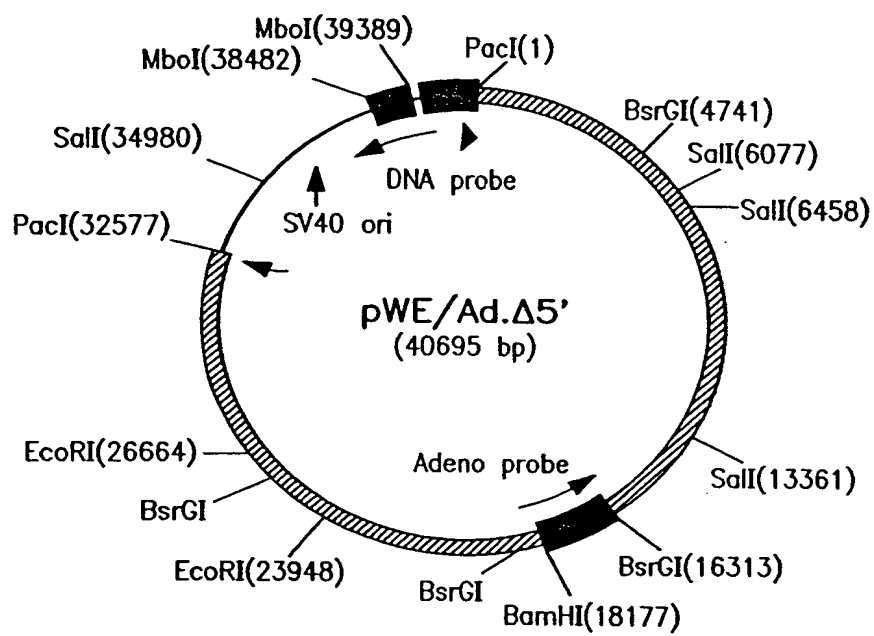
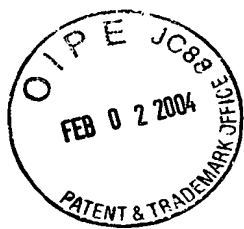


FIG. 26A

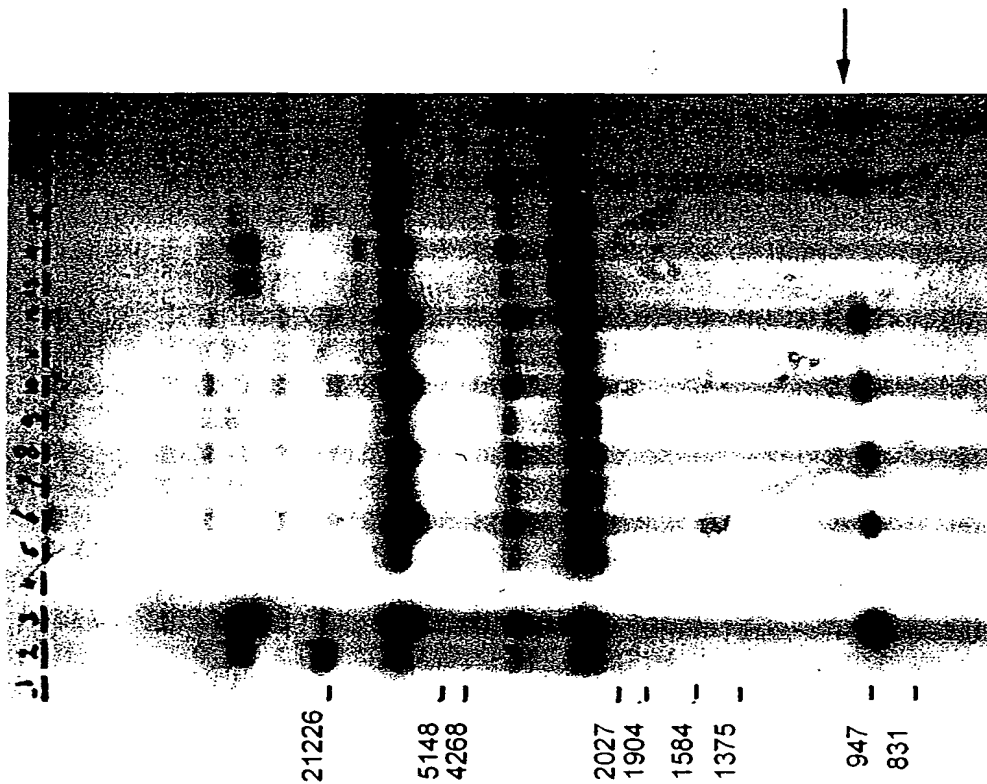
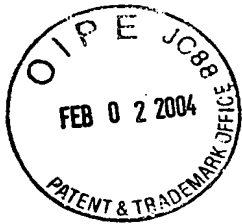


FIG. 26C

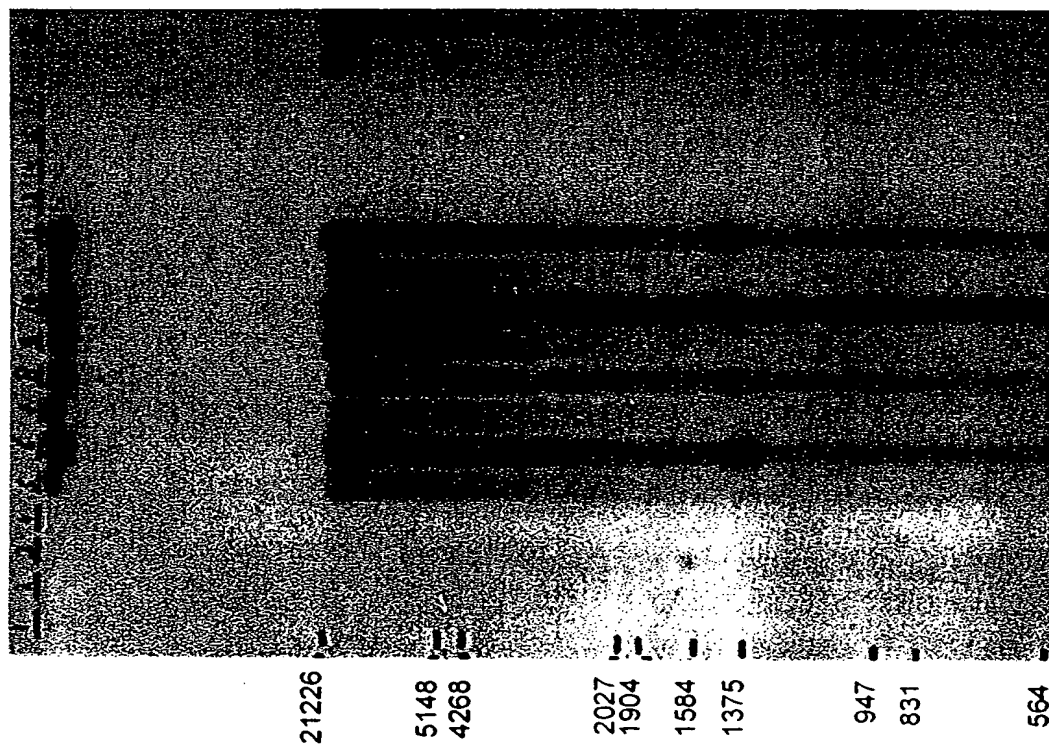
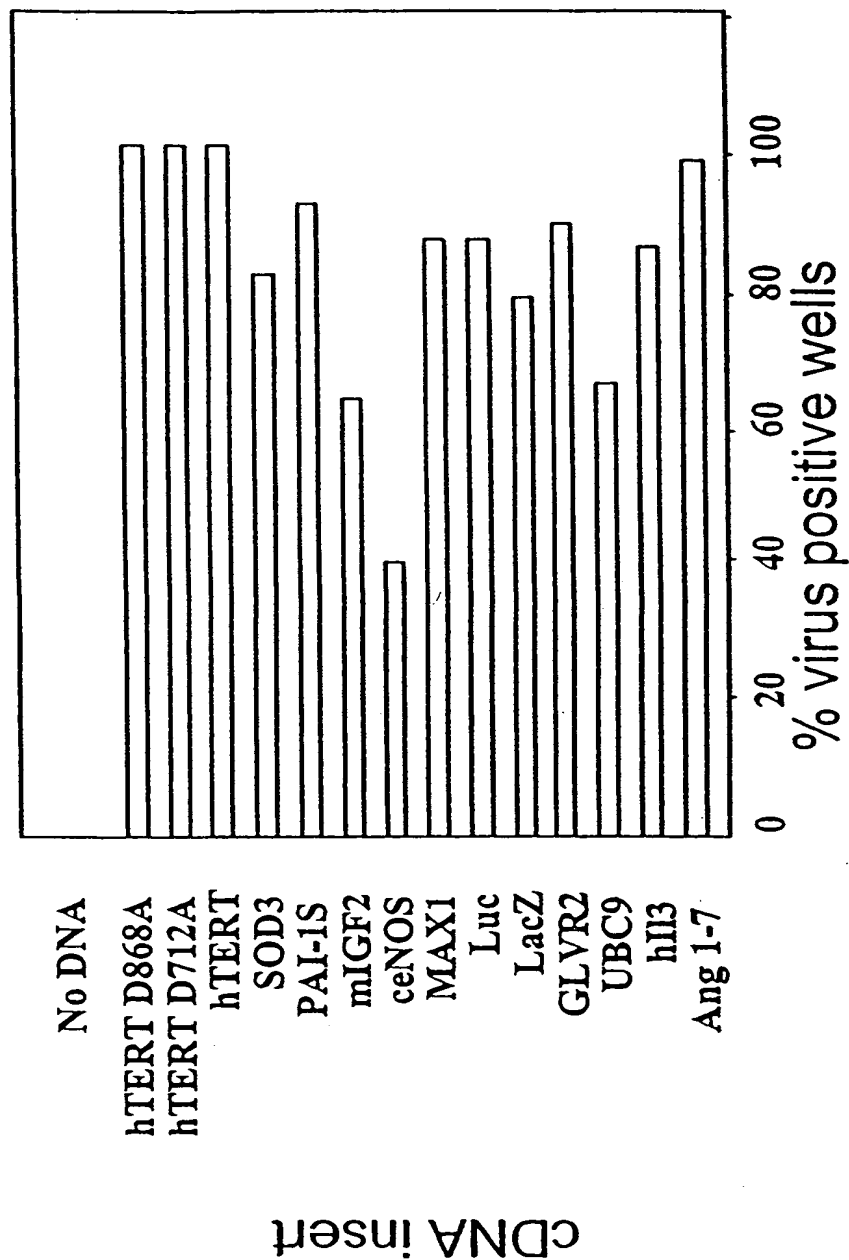
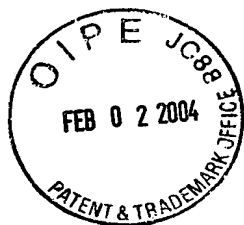


FIG. 26B



Average percentage CPE efficiency: 86 %

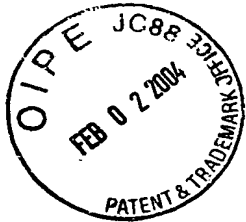
FIG. 27



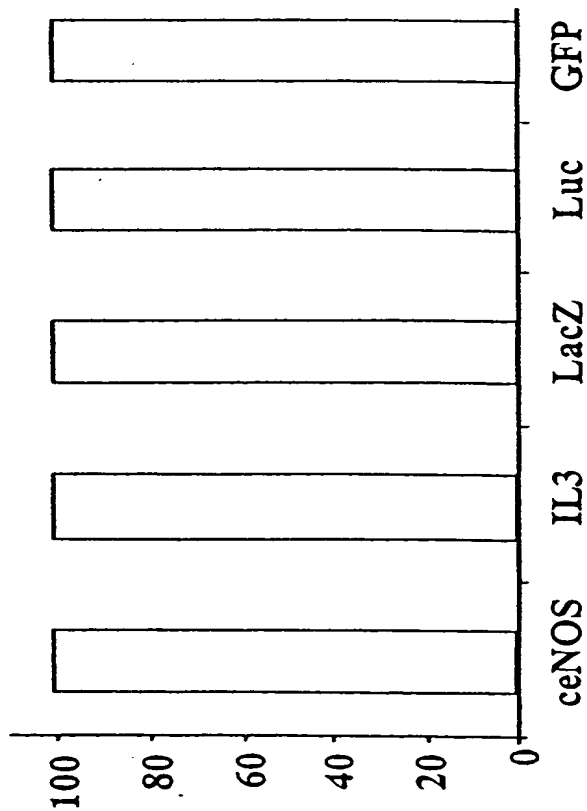
Gene	Insert kb
• ceNOS	3.6
• hTERT	3.5
hTERT D712A	3.5
lacZ	3.2
hCAT1	2.2
• GLVR2	2.0
• Luc	1.7
• SOD3	1.4
• MAX1	.550
• hVEGF121	.511
• hIL3	.434
• UBC9	.412
ANG1-7	.104

Average titer  
 $0.8 \pm 0.7 \times 10^9$  pfu/ml

FIG. 28



% wells producing functional virus



Gene	Number of CPE+ wells
ceNOS	19/19
IL3	7/7
lacZ	36/36
Luc	40/40
GFP	48/48

Gene	Number of plaques
ceNOS	9/9
IL3	9/9
lacZ	40/40
Luc	9/9
EGFP	IP
GLVR2	9/9

FIG. 29



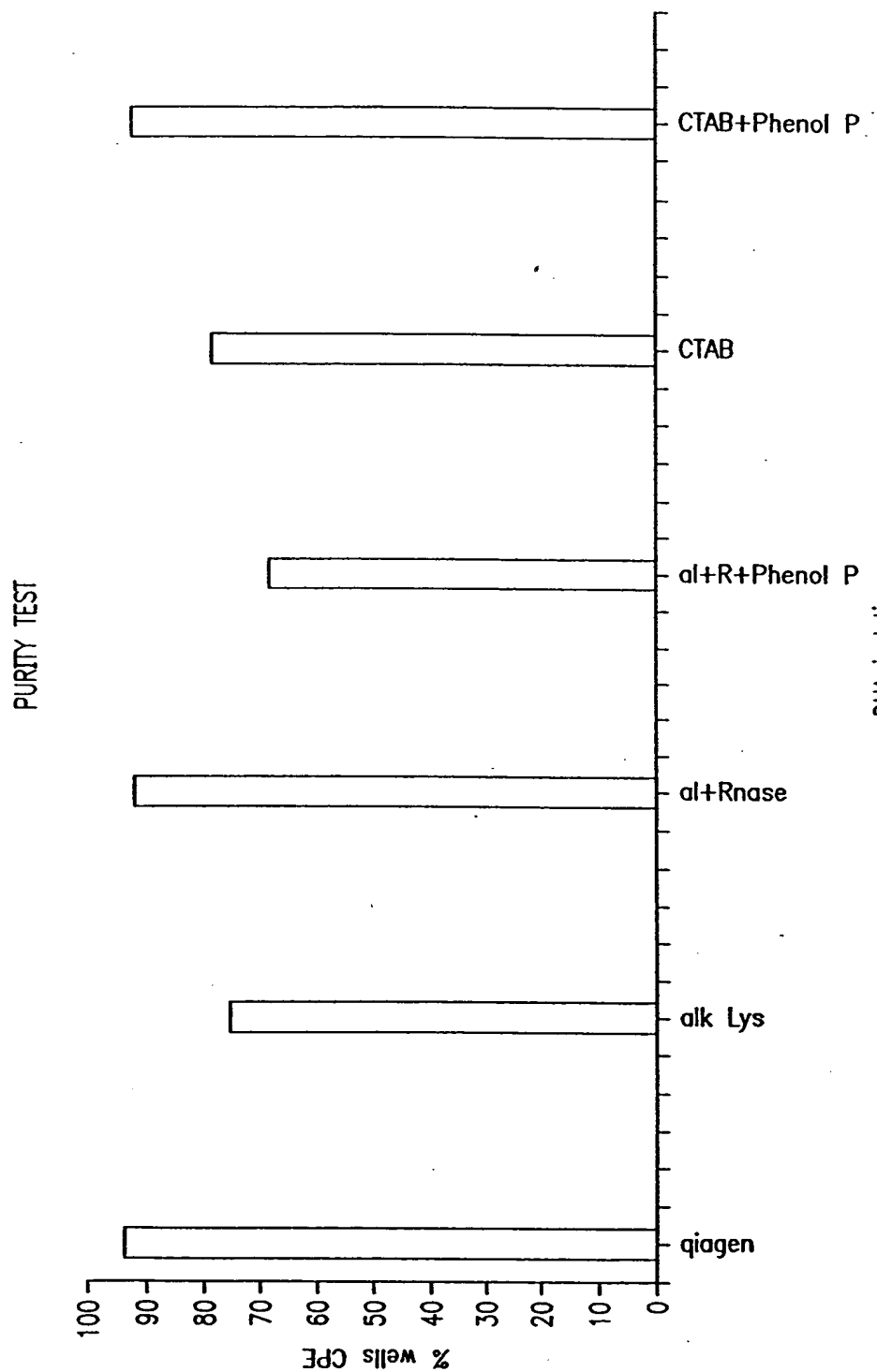
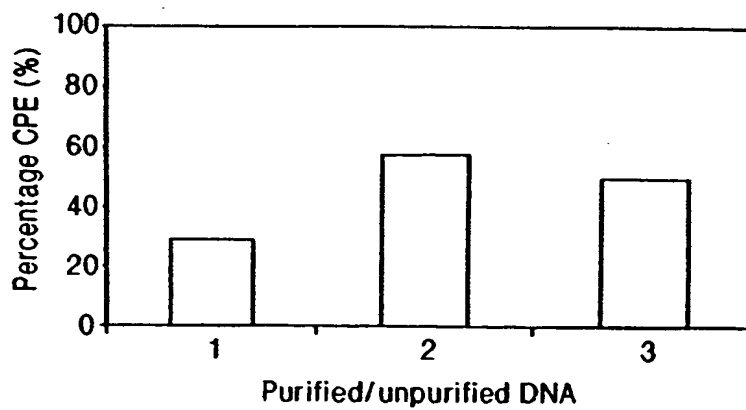
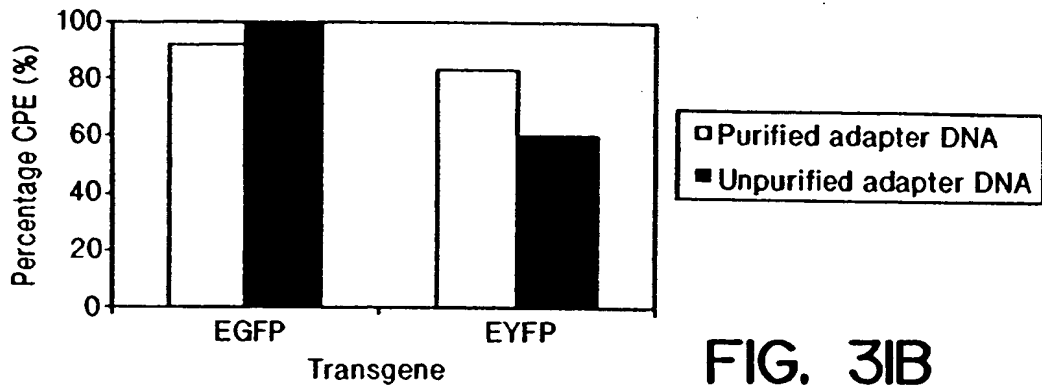
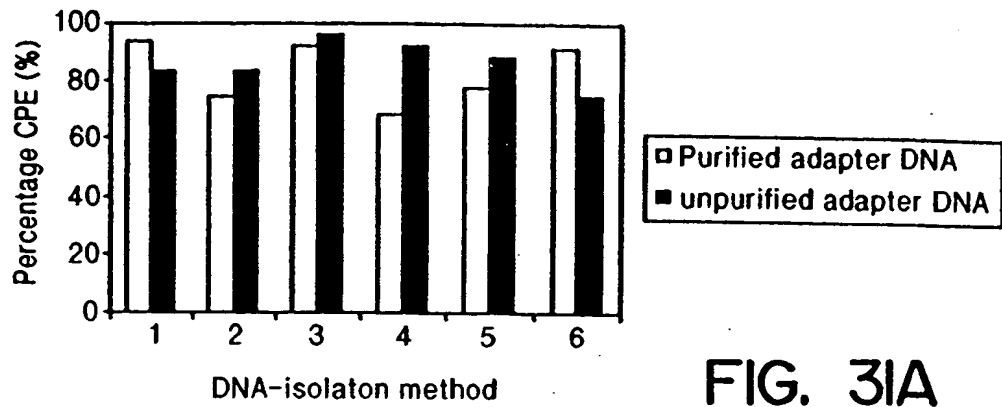


FIG. 30



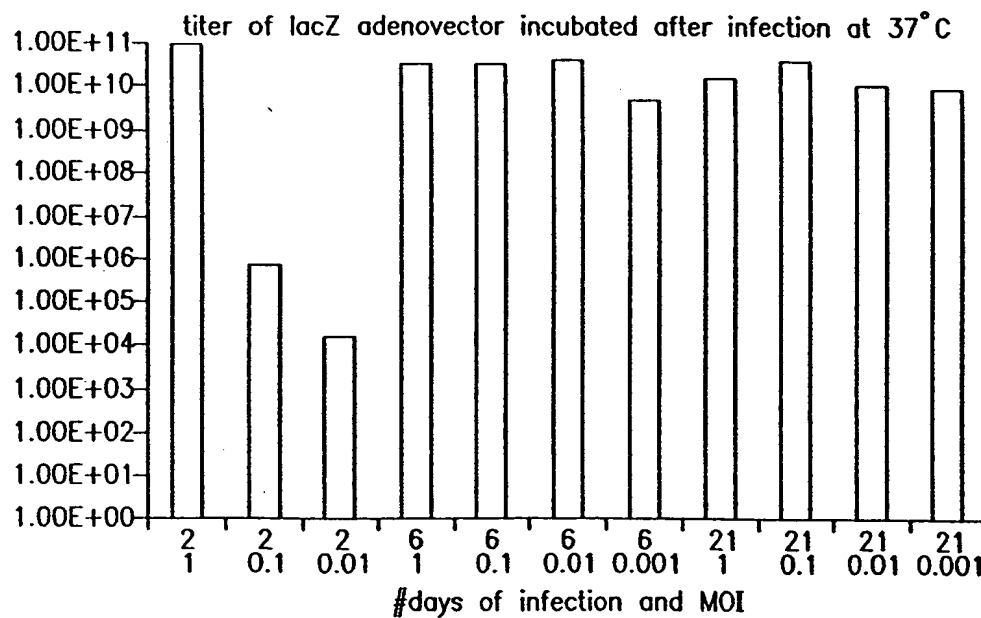
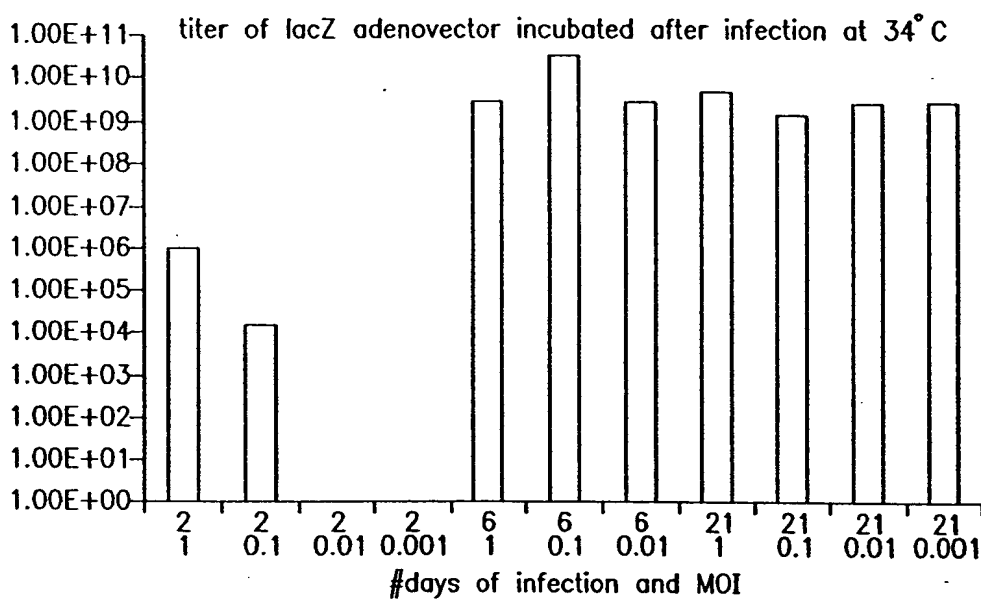
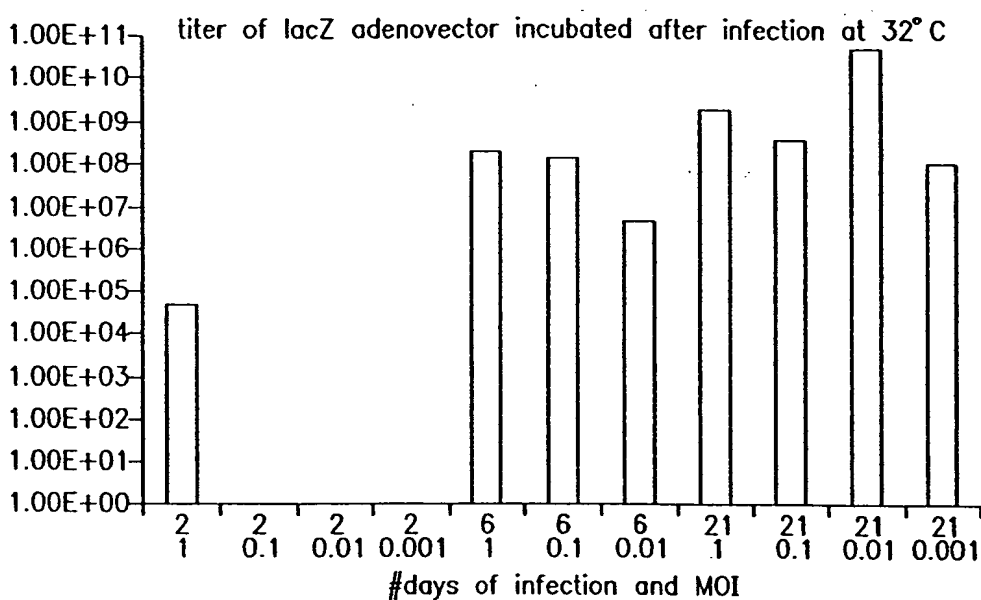


FIG. 32

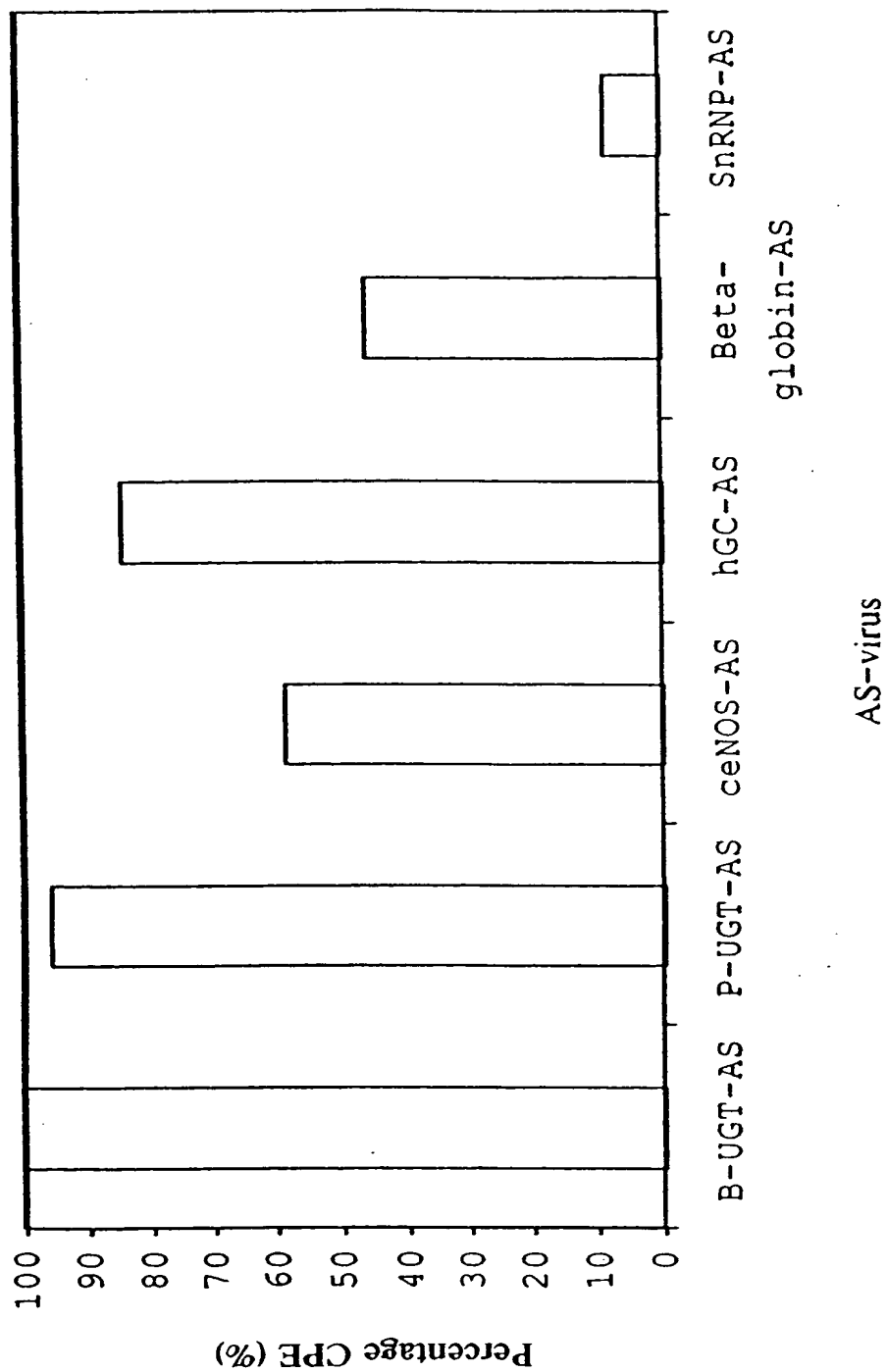
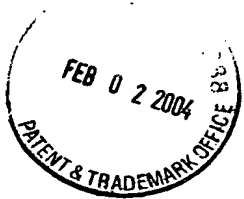


FIG. 33

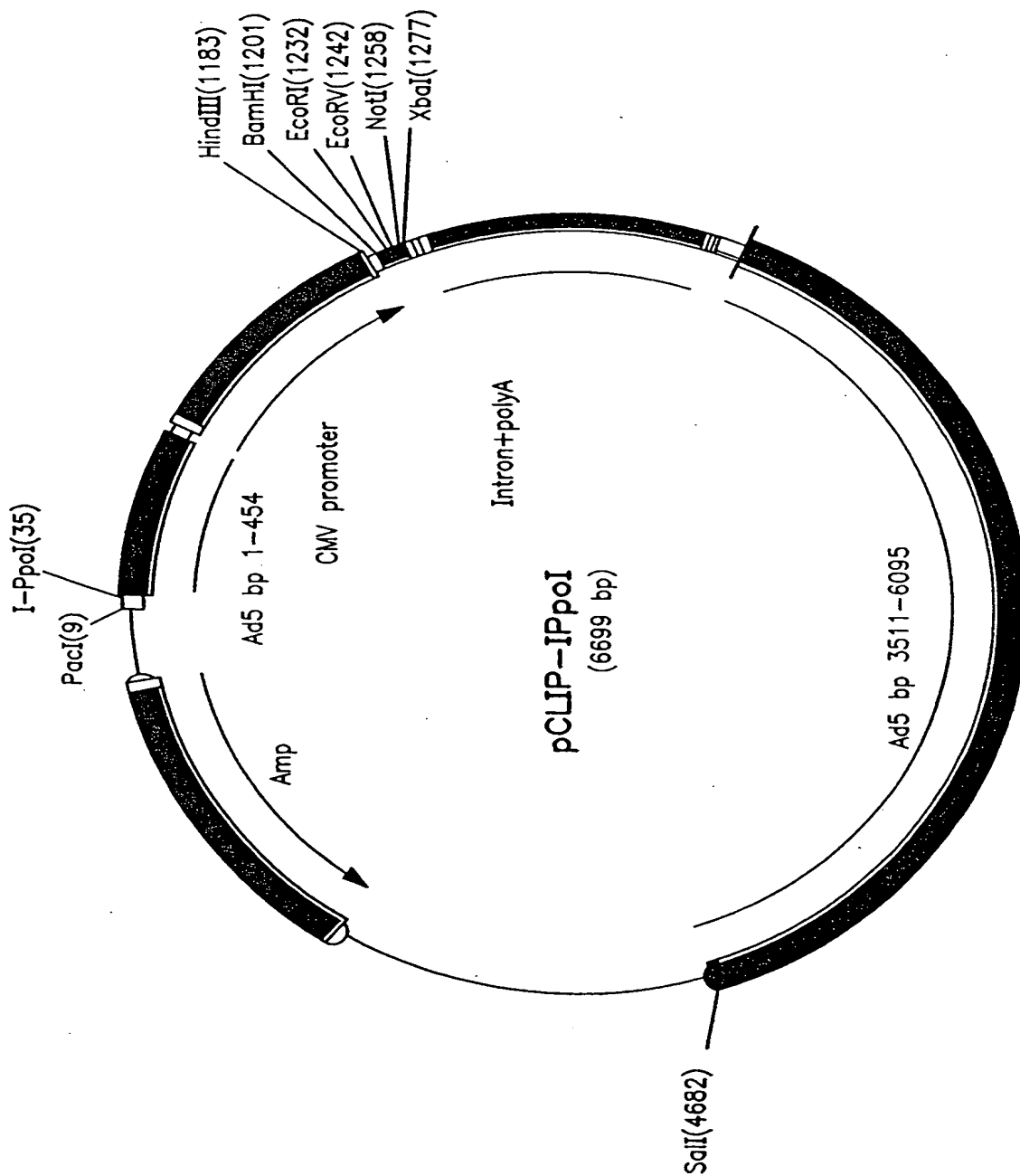


FIG. 34A

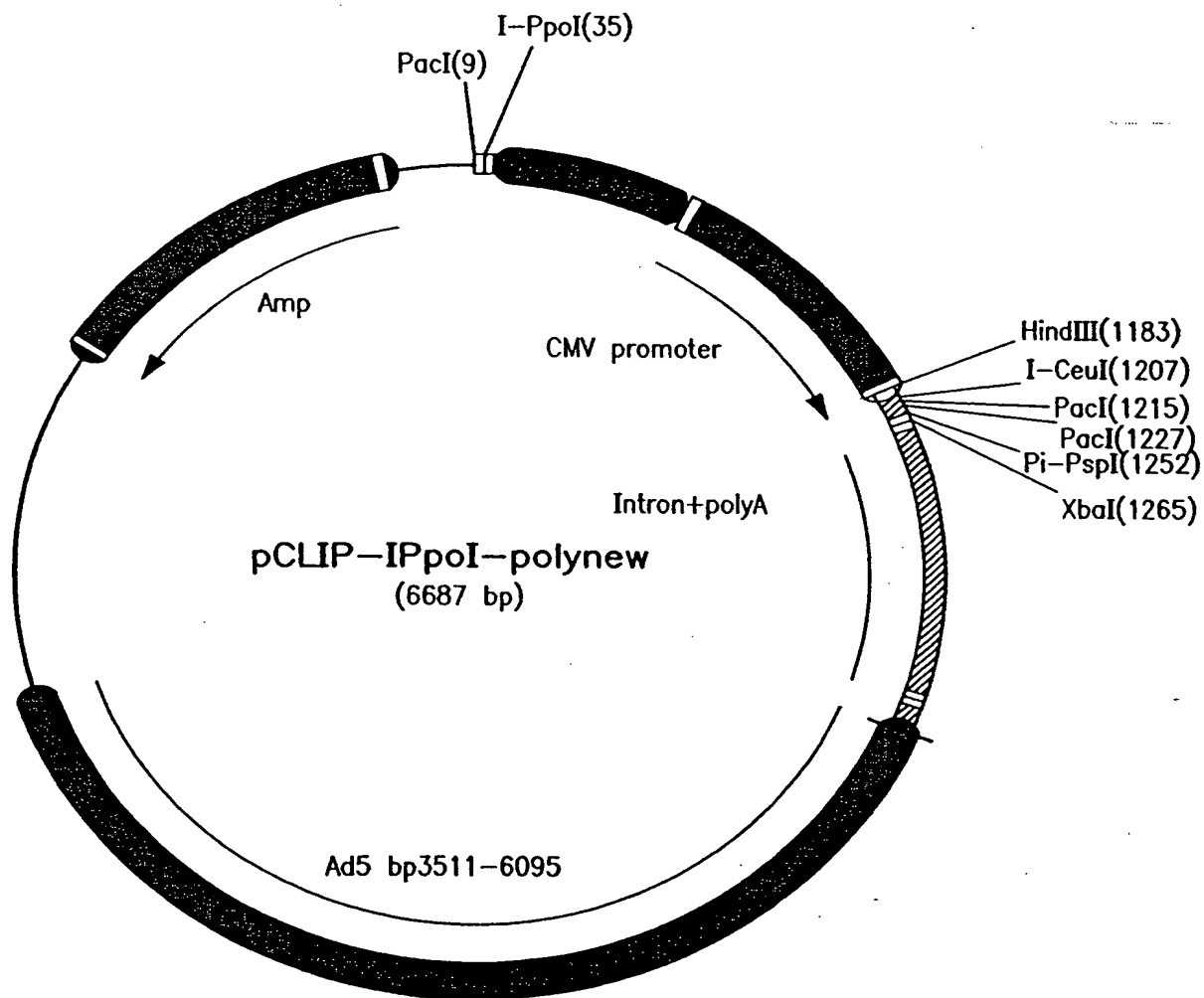


FIG. 34B

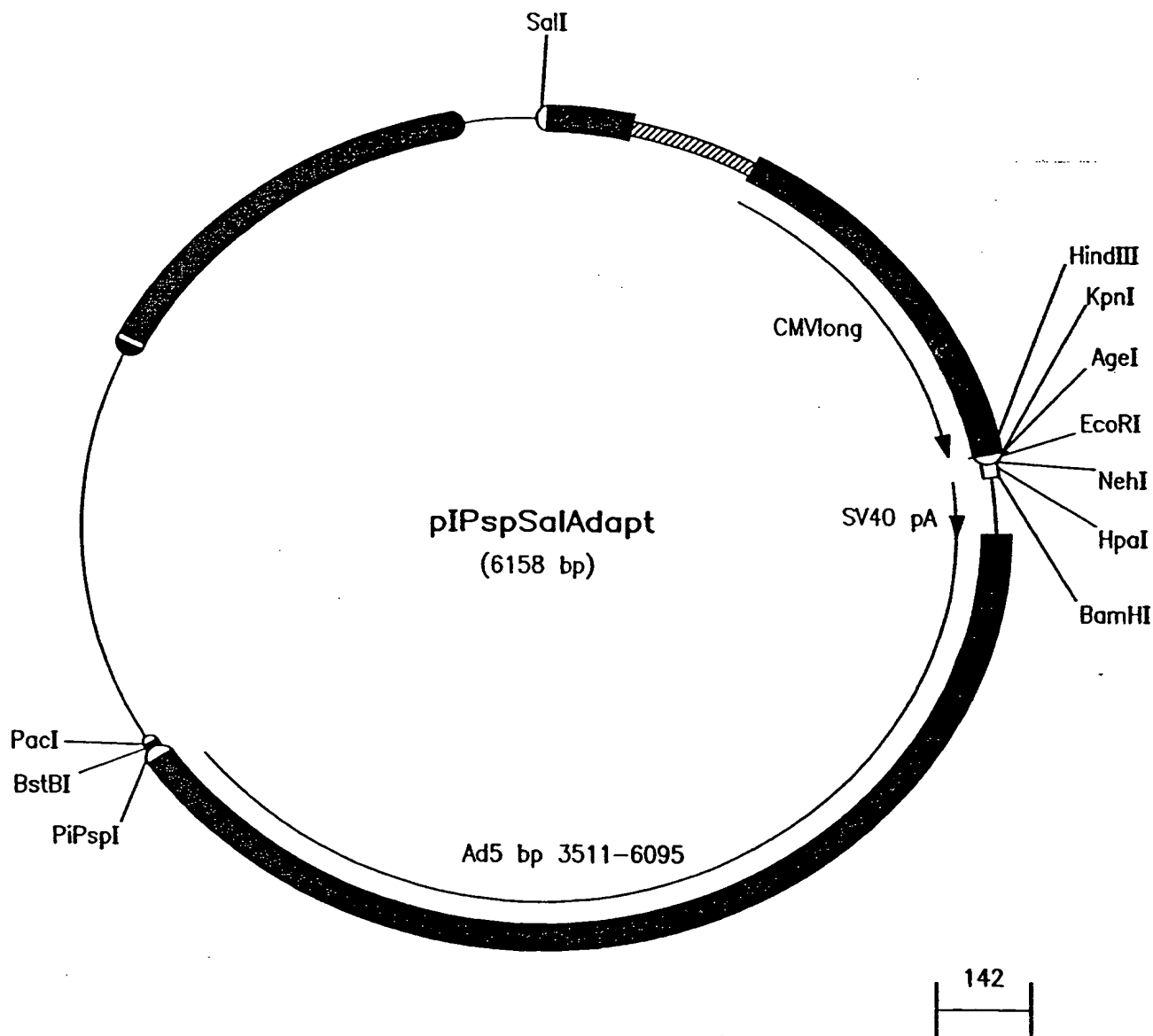
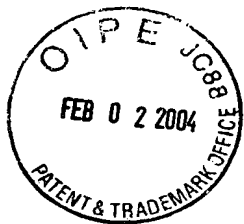


FIG. 34C

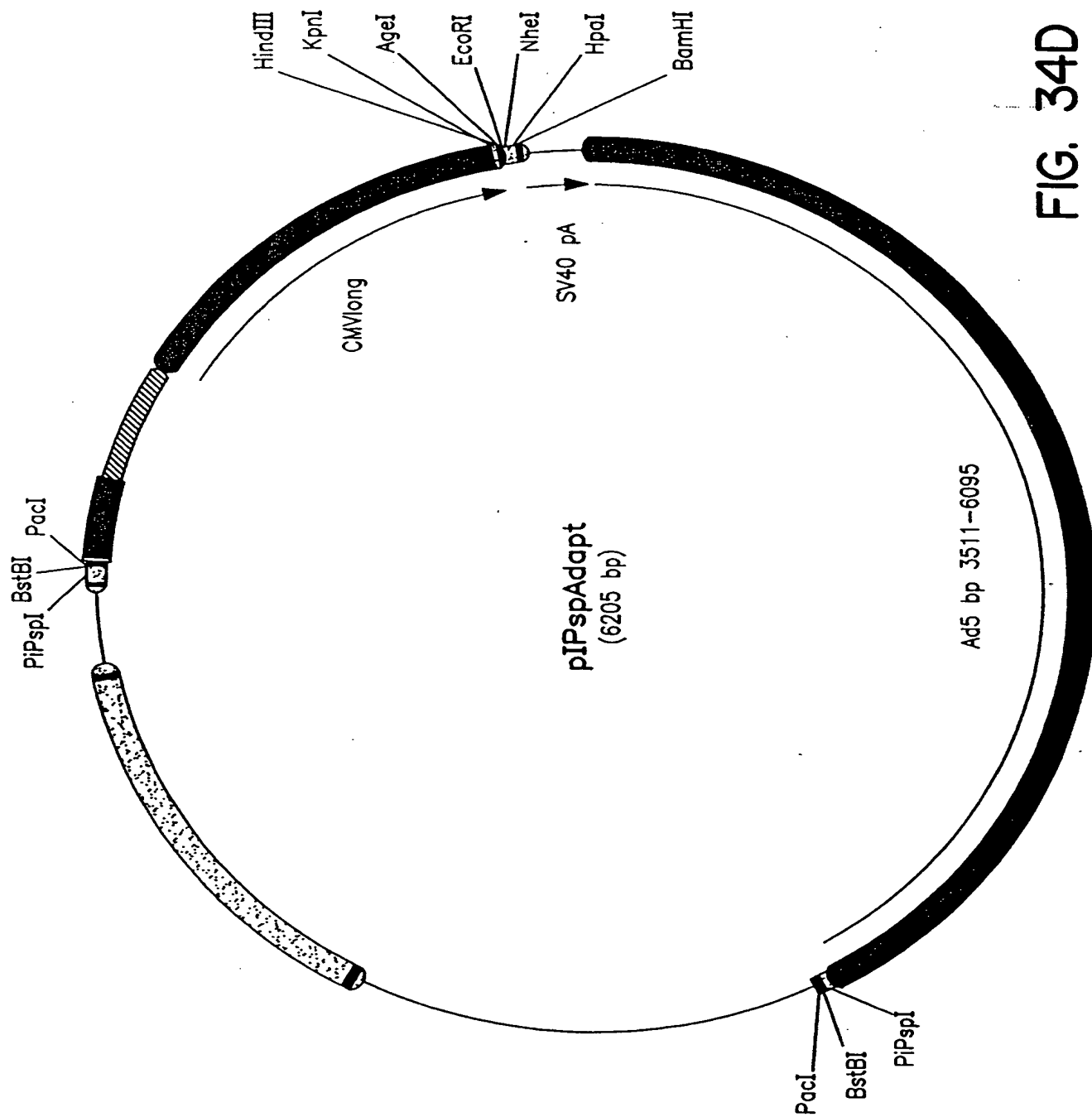
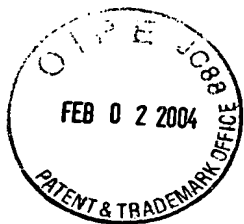


FIG. 34D



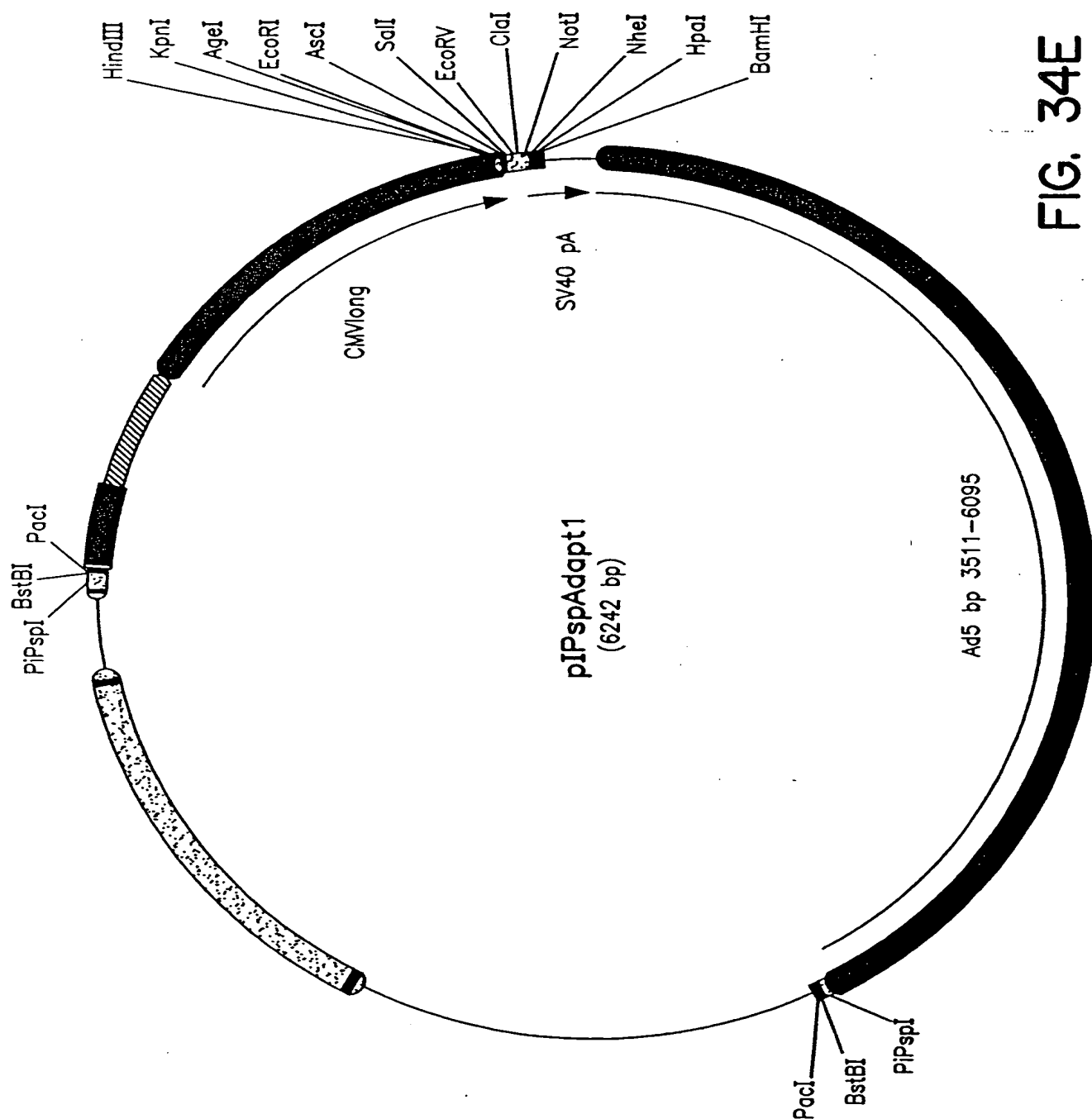
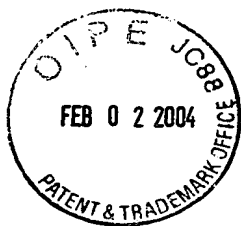


FIG. 34E

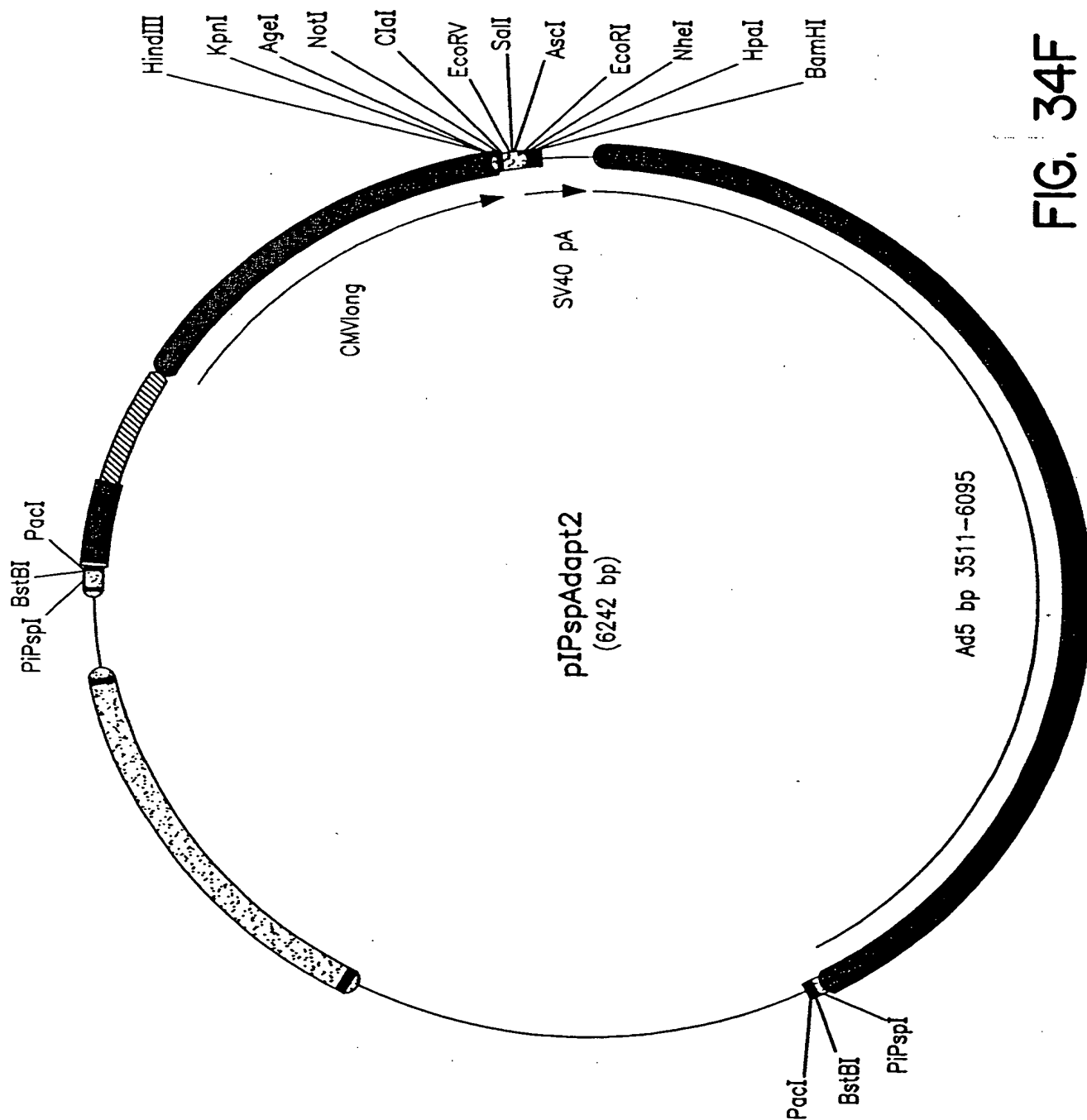
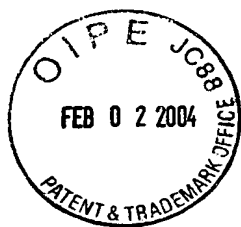


FIG. 34F

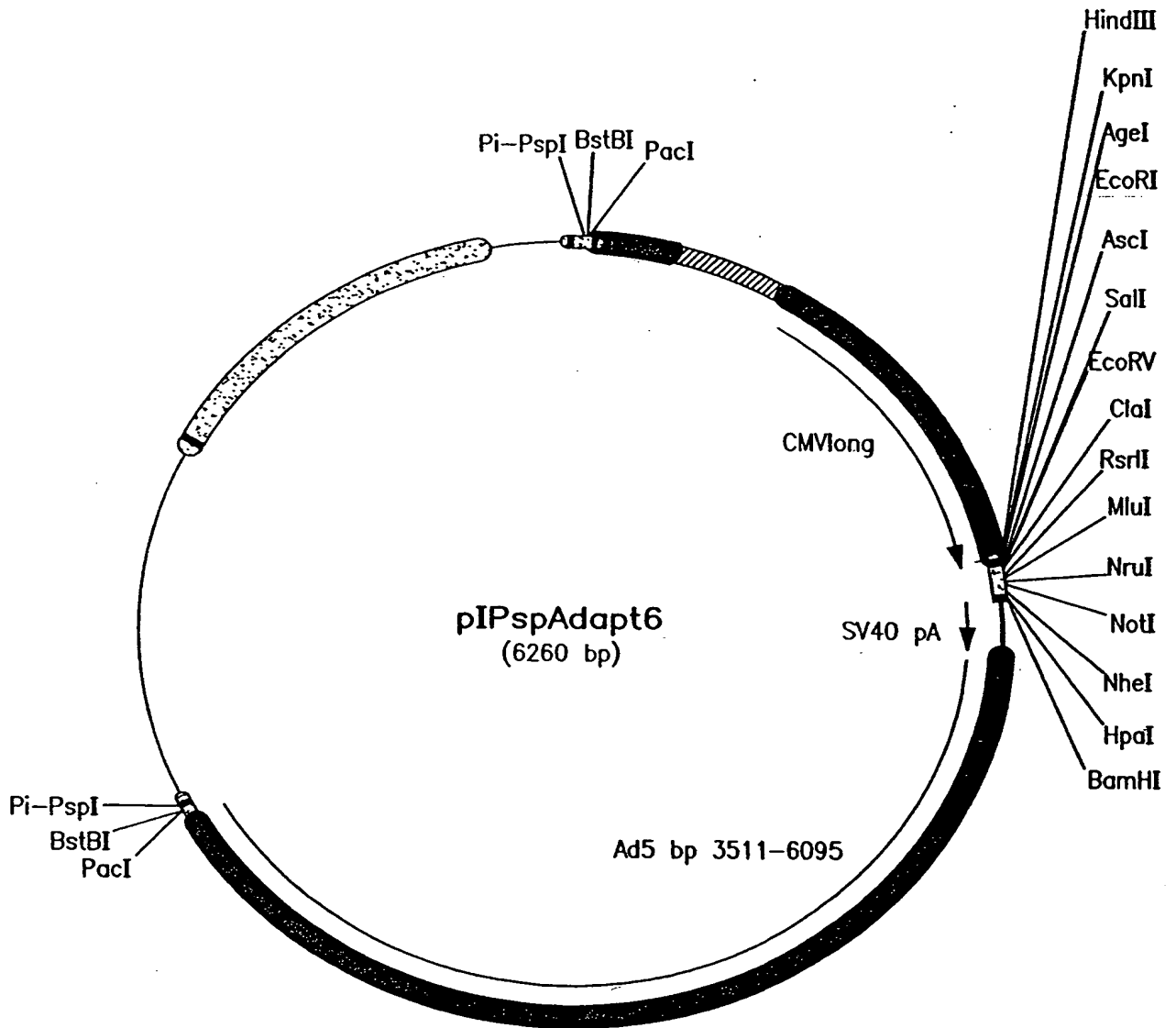


FIG. 34G

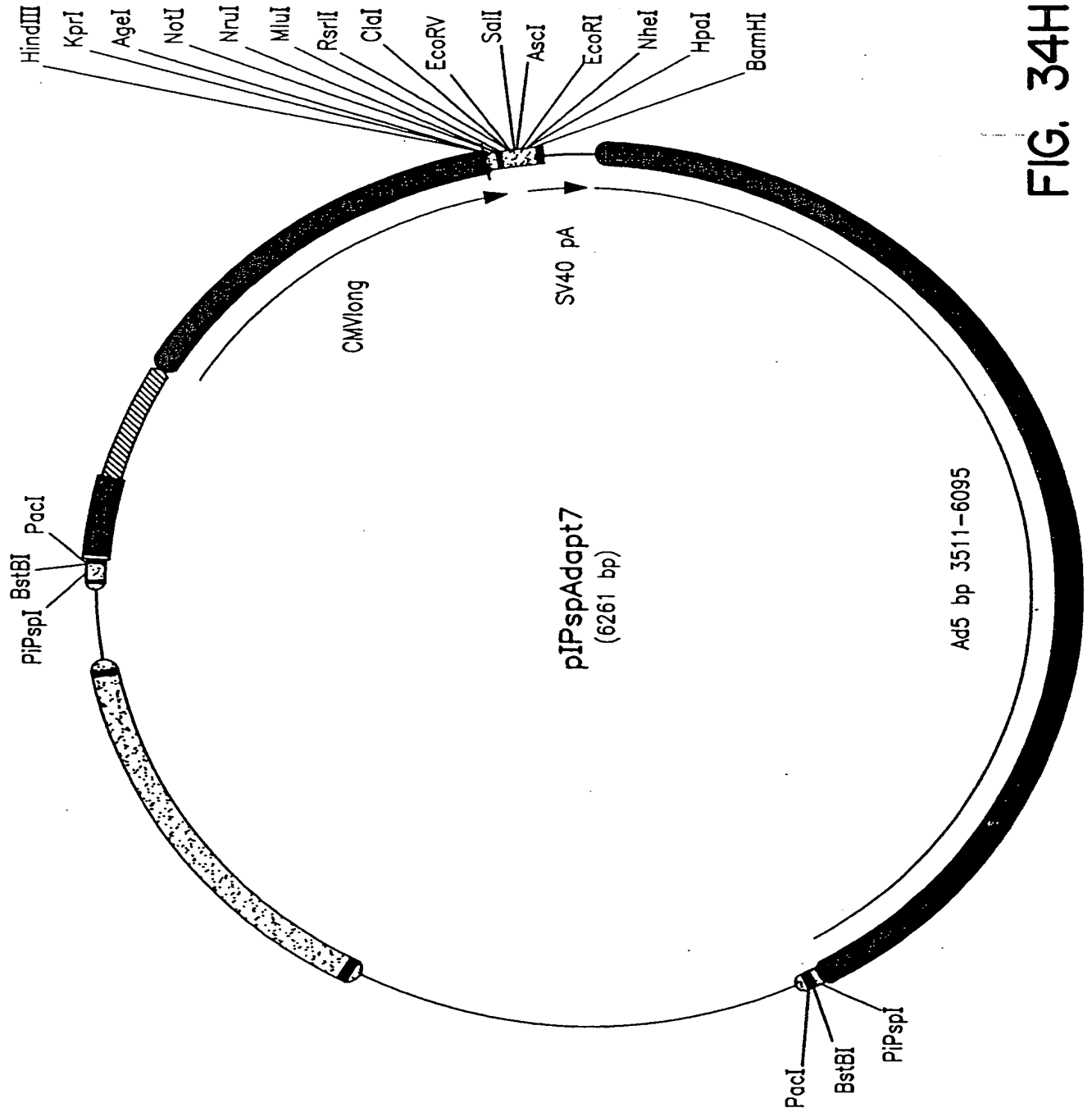
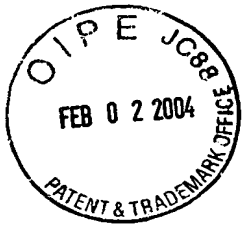


FIG. 34H

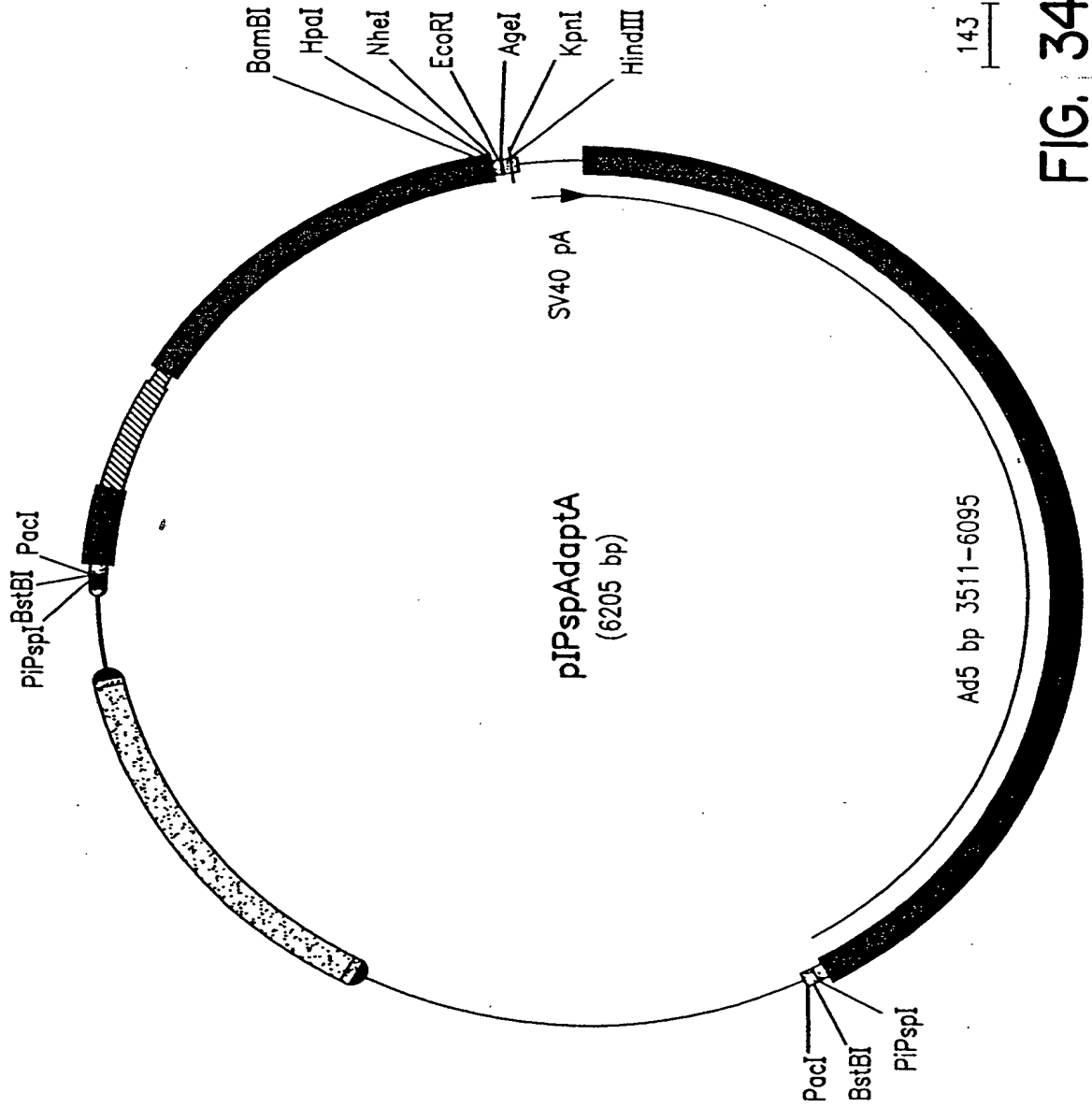
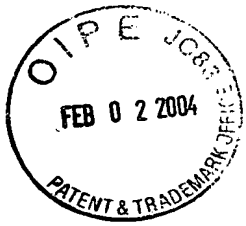


FIG. 34 I

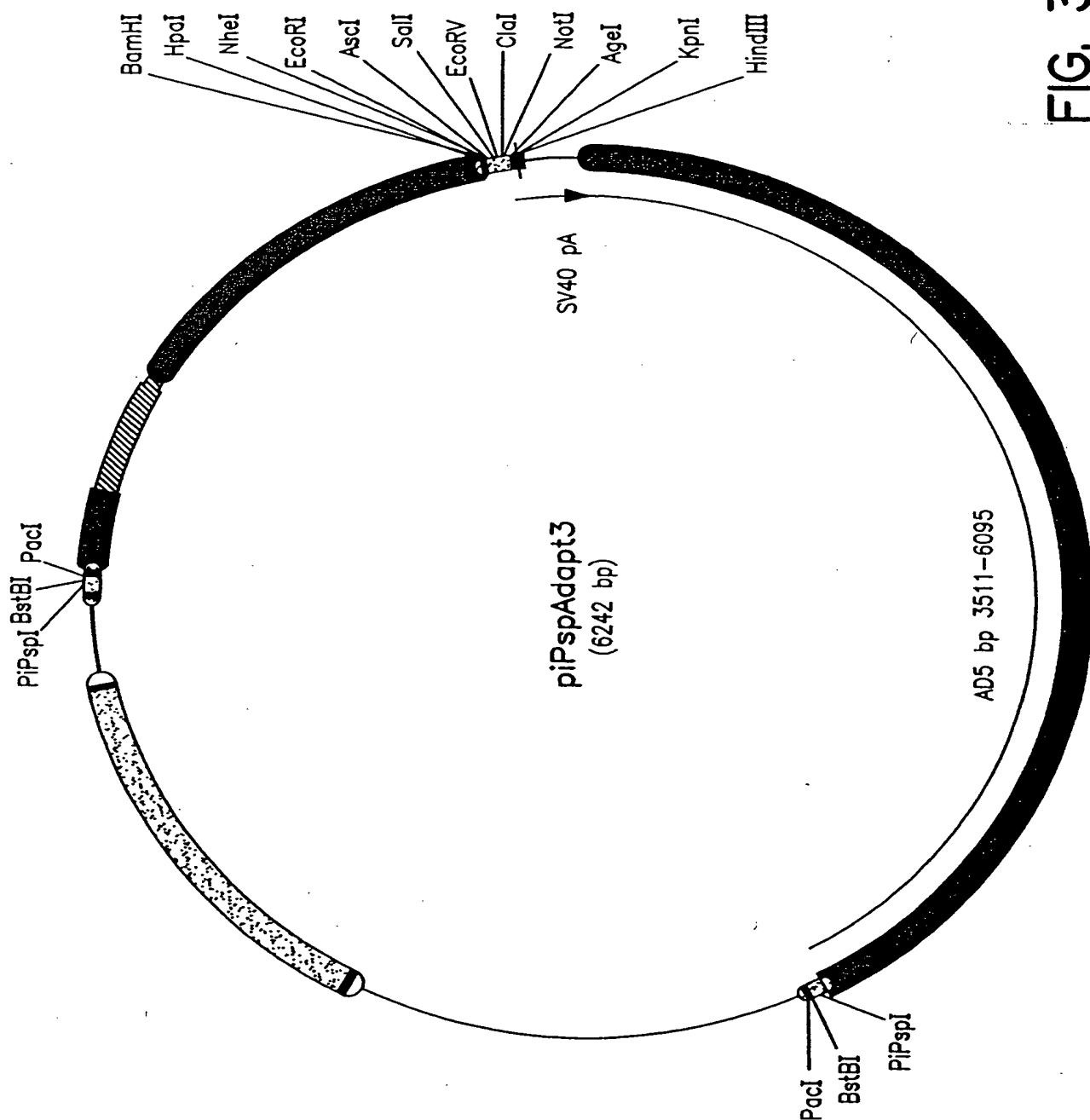
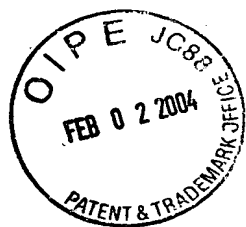


FIG. 34J

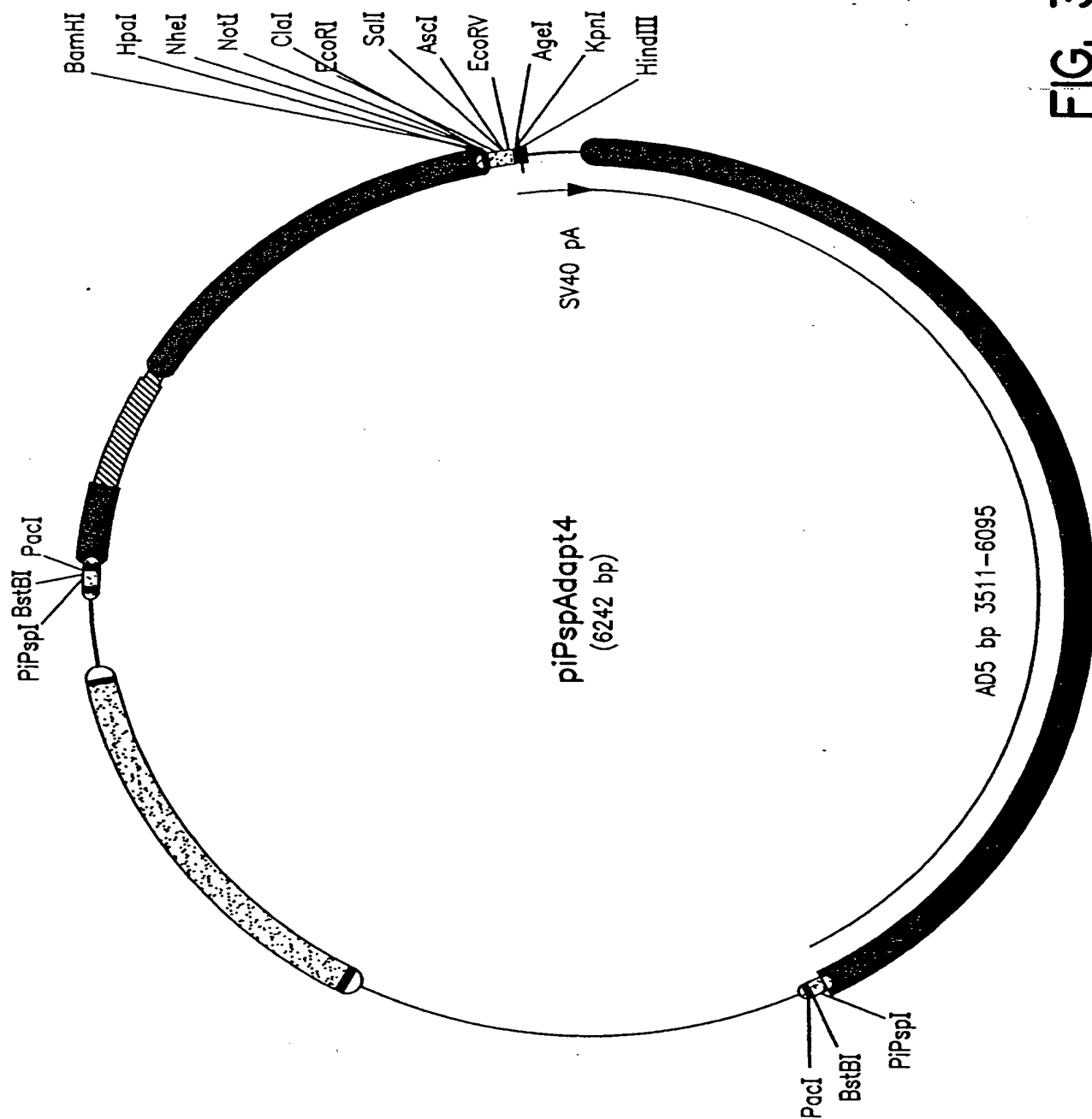
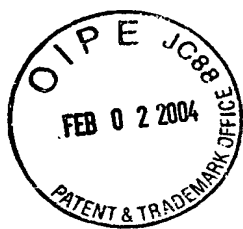


FIG. 34K

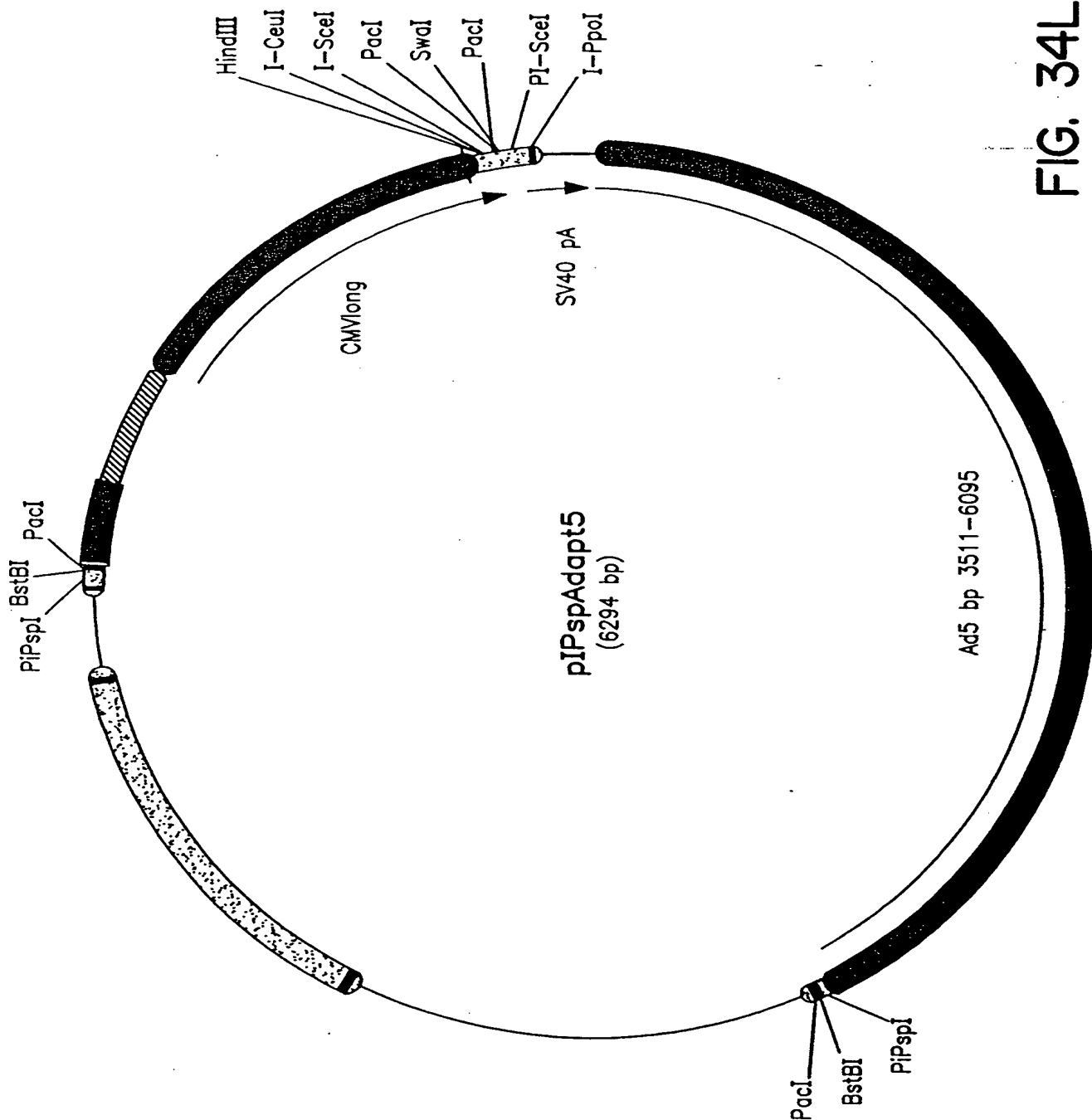


FIG. 34L



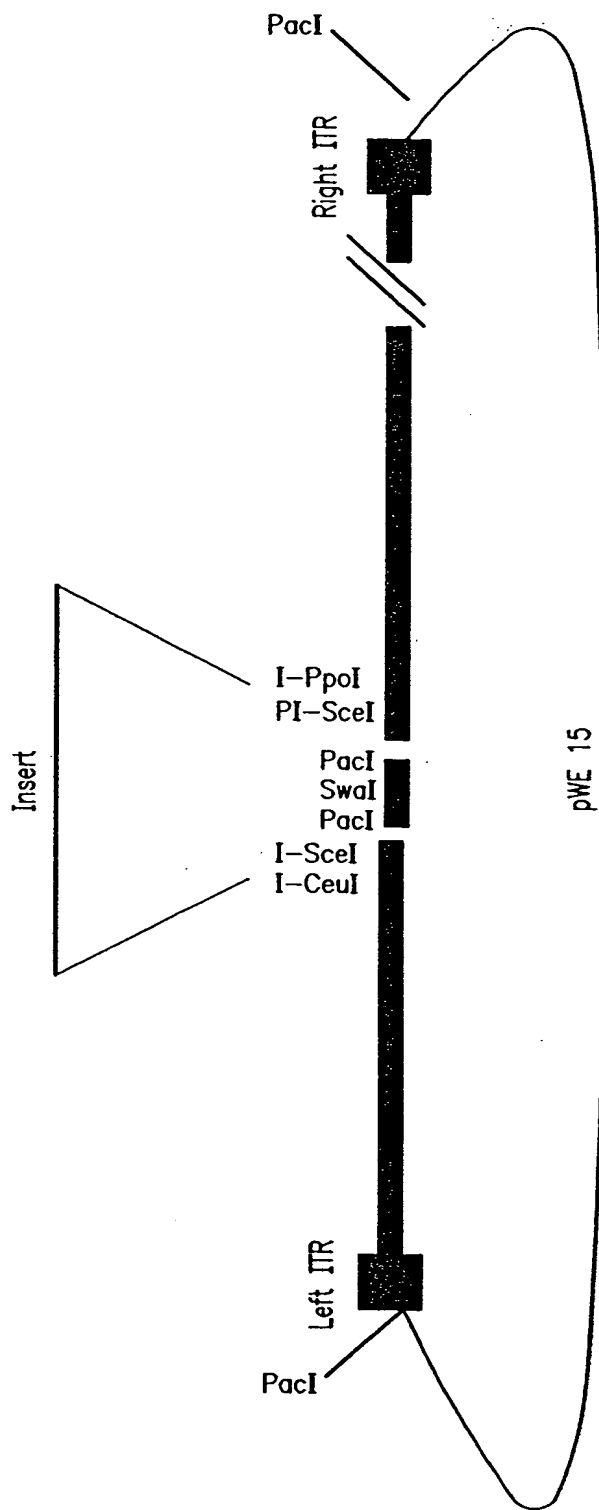


FIG. 34M



Relative amounts of wells with CPE after transfection of PER.C6/E2A cells with pCLIP-LacZ and the adapter plasmid pIPspAdapt2.

Transfection of pIPspAdapt2 to PER.C6/E2A

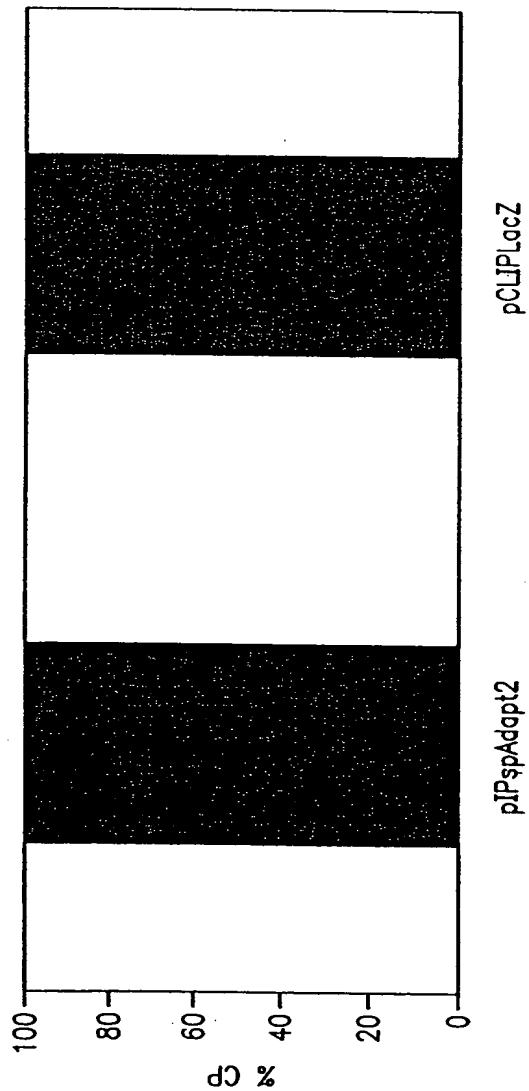


FIG. 34N

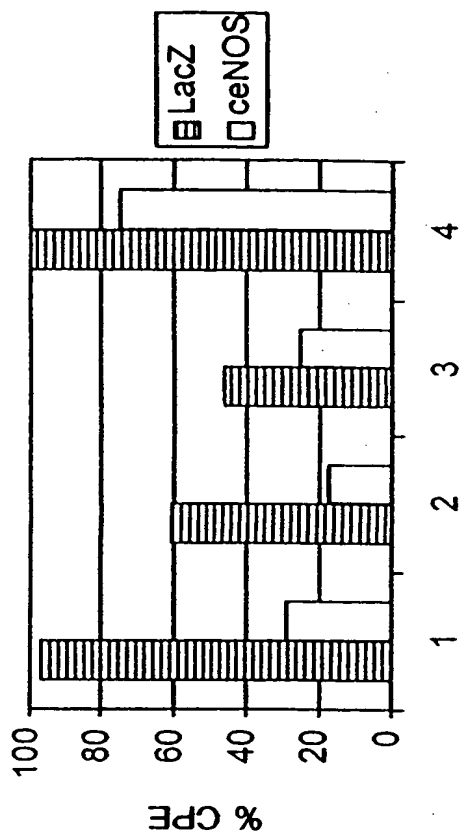


FIG. 35



Construction total Adeno cDNA Library (1)

Cells/tissue → mRNA isolation → cDNA → E.coli transformation

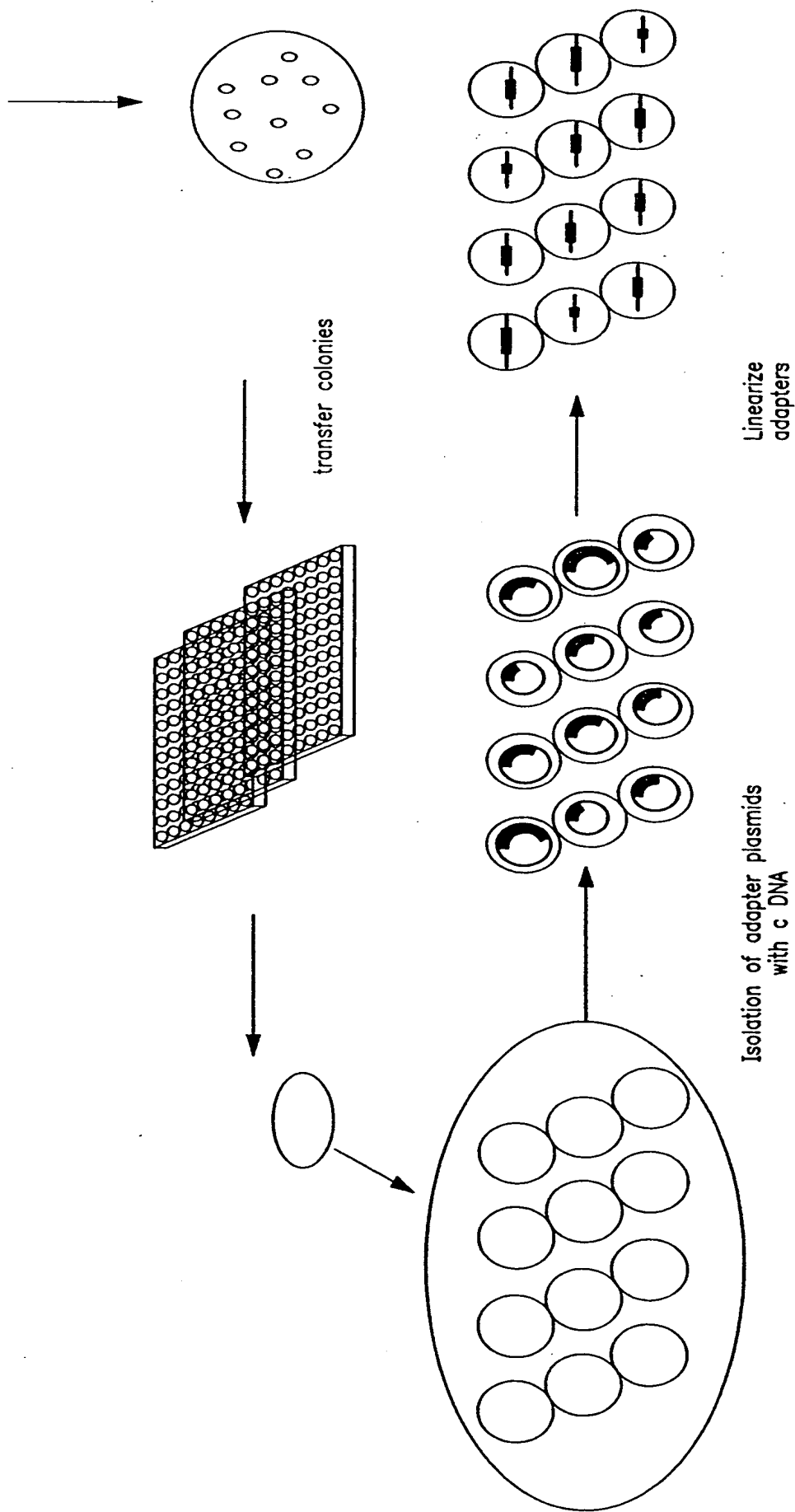


FIG. 36A



# Construction total Adeno cDNA Library (II)

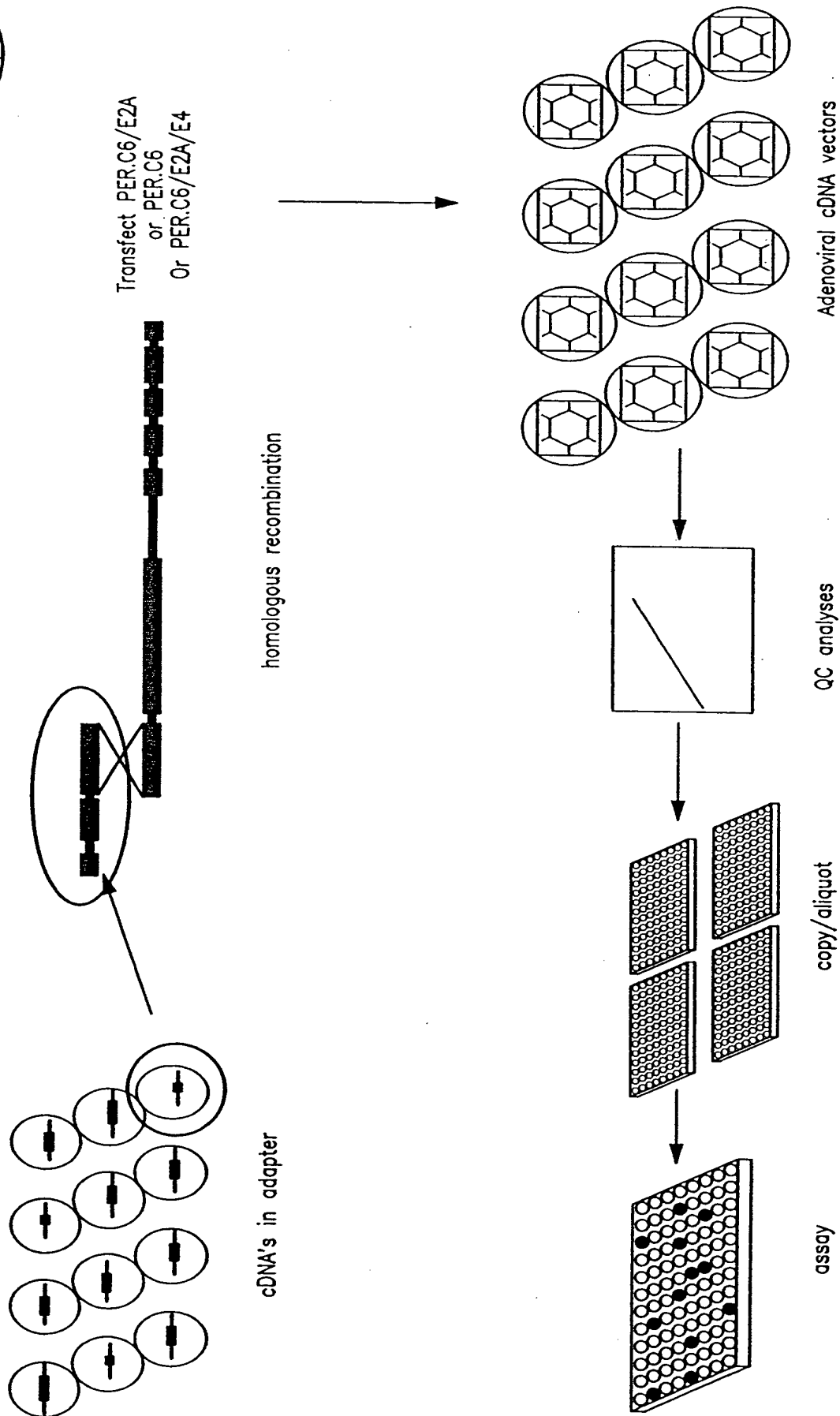
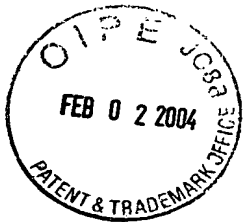


FIG. 36B



EXAMPLE 21. 384 WELL PLATE IN PROGRESS

Co-transfections on 384 well plates

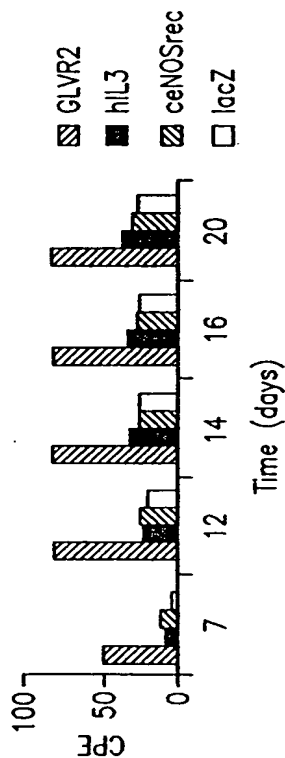


FIG. 37A

Co-transfections on 96 well plates  
(control plate)

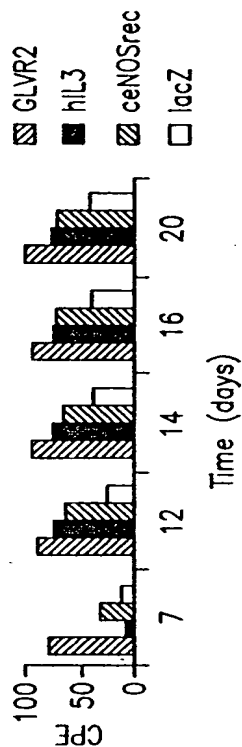


FIG. 37B

Co-transfections on 384 well plates

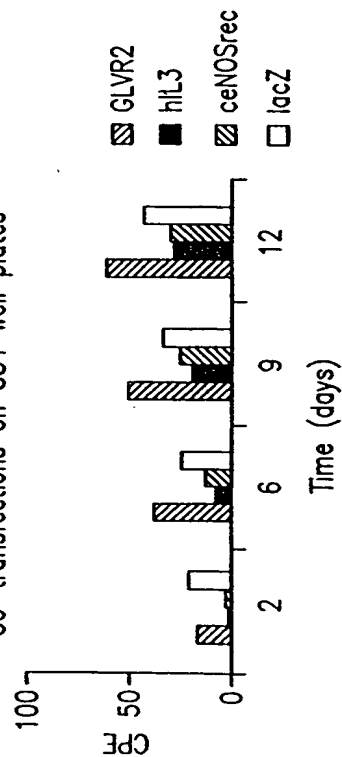


FIG. 37C

Co-transfections on 96 well plates  
(control plate)

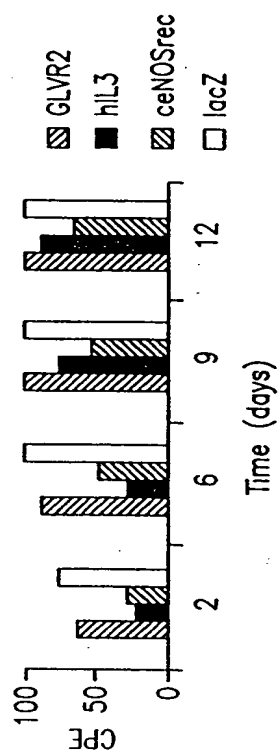


FIG. 37D



Medium changed 7 days after transfection

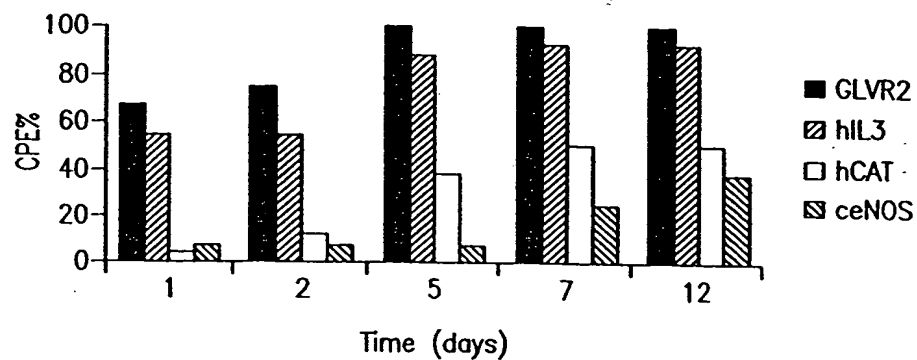


FIG. 38A

Medium not changed

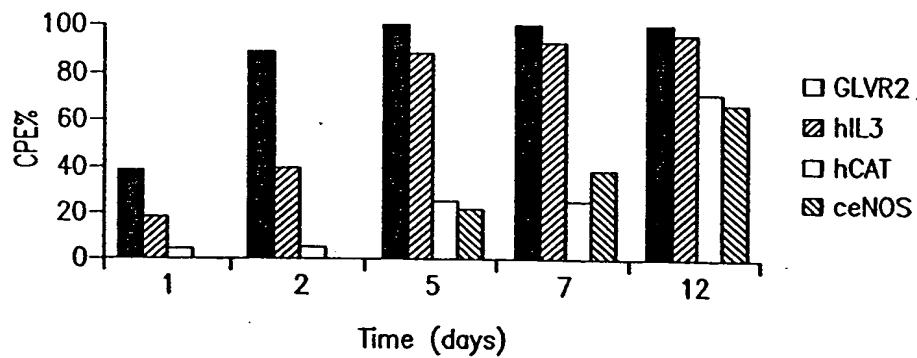


FIG. 38B

Propagation 7 days after transfection

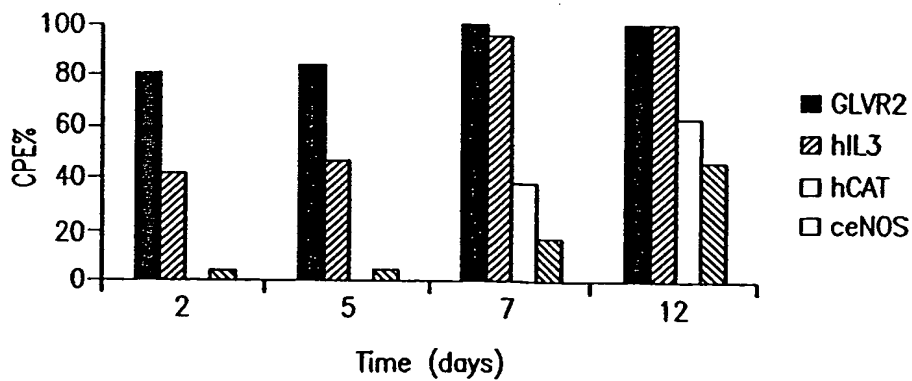


FIG. 38C



Cell titration experiment #1

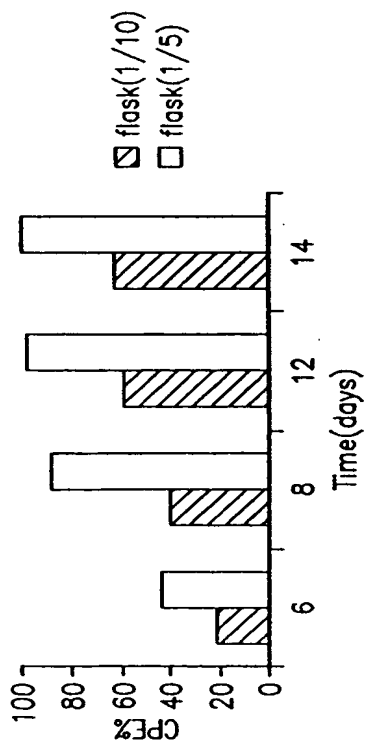


FIG. 39A

Cell titration experiment #2

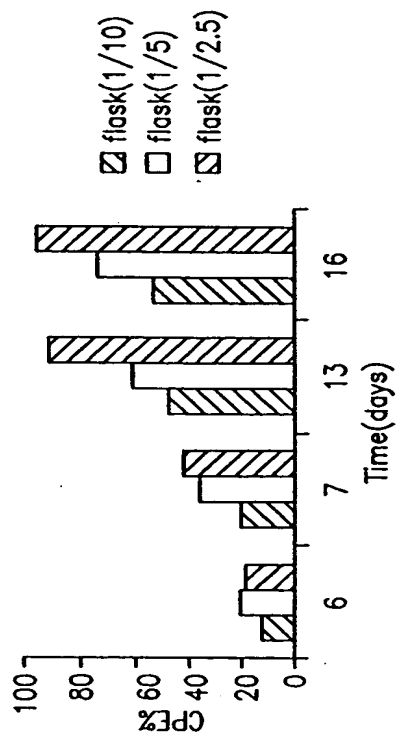


FIG. 39B

Cell titration experiment #3

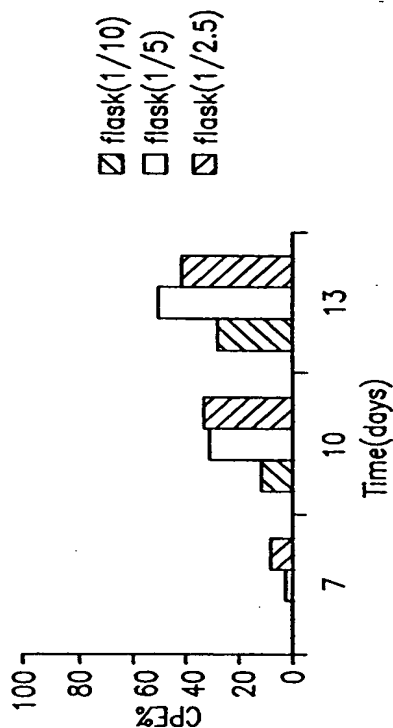


FIG. 39C



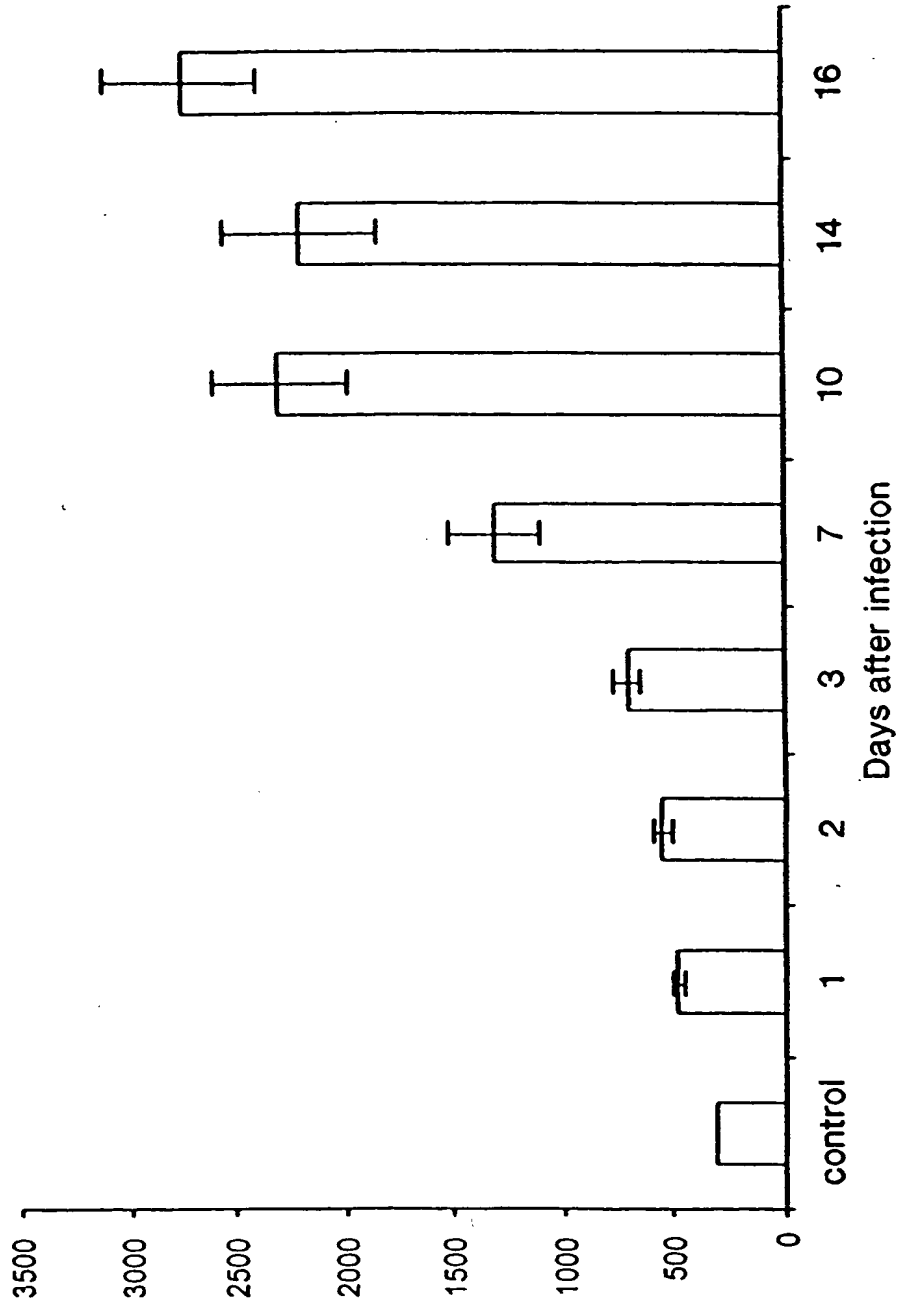
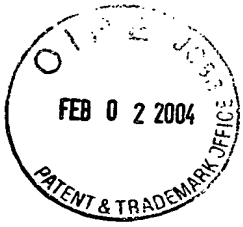


FIG. 40

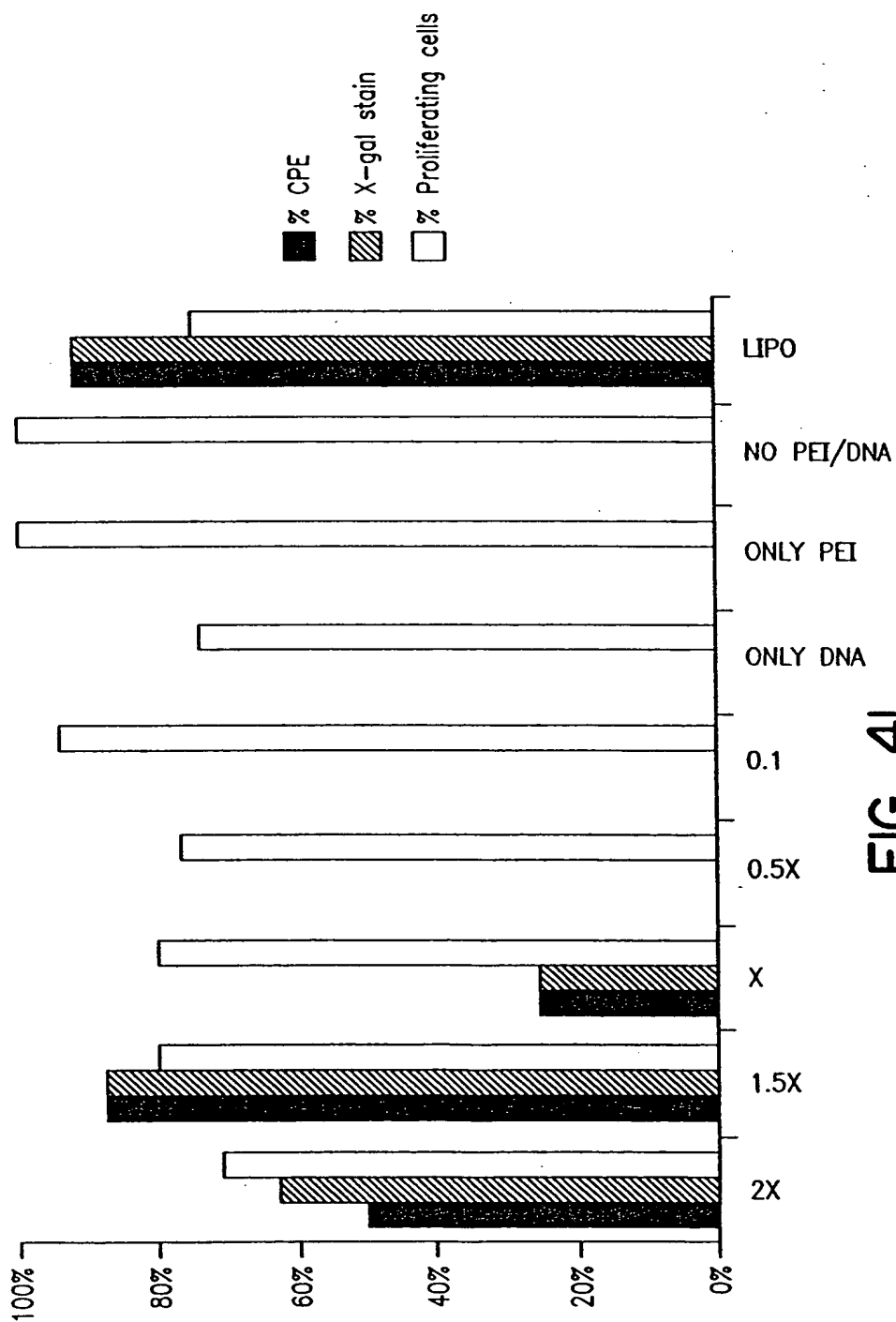


FIG. 4I

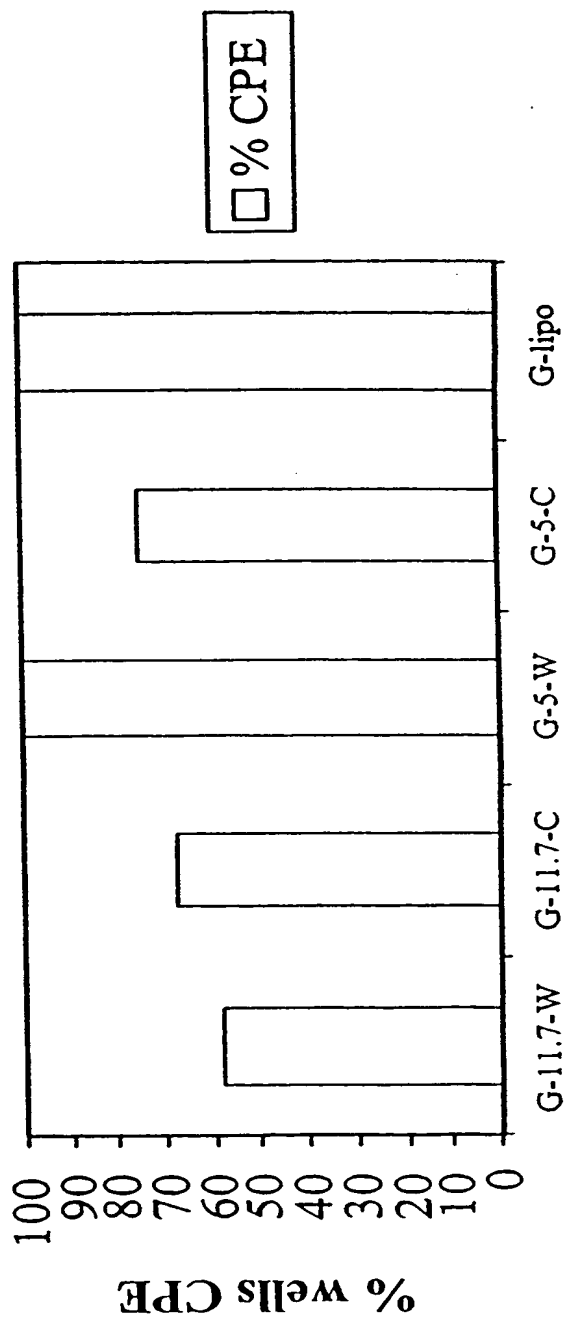


FIG. 42

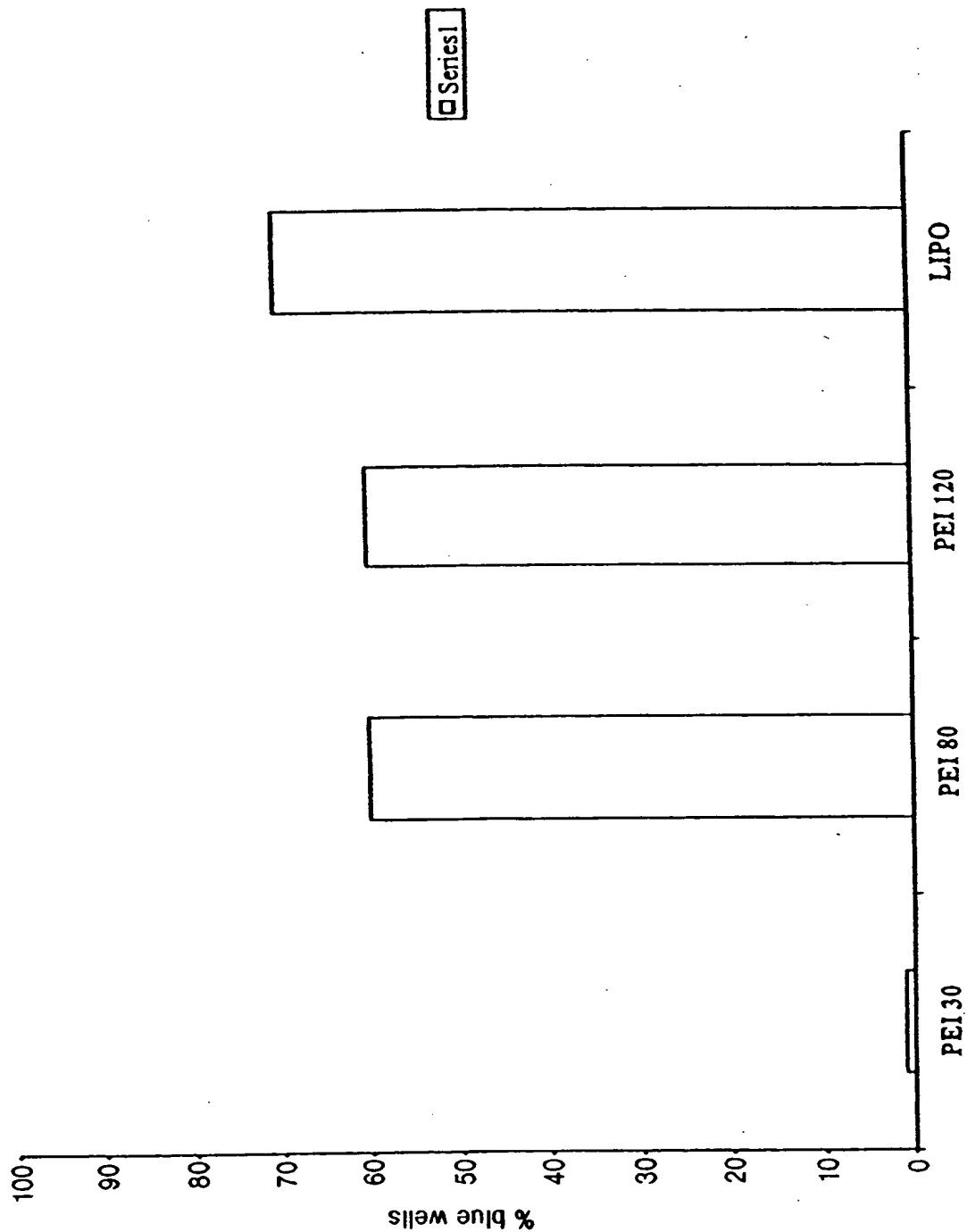


FIG. 43

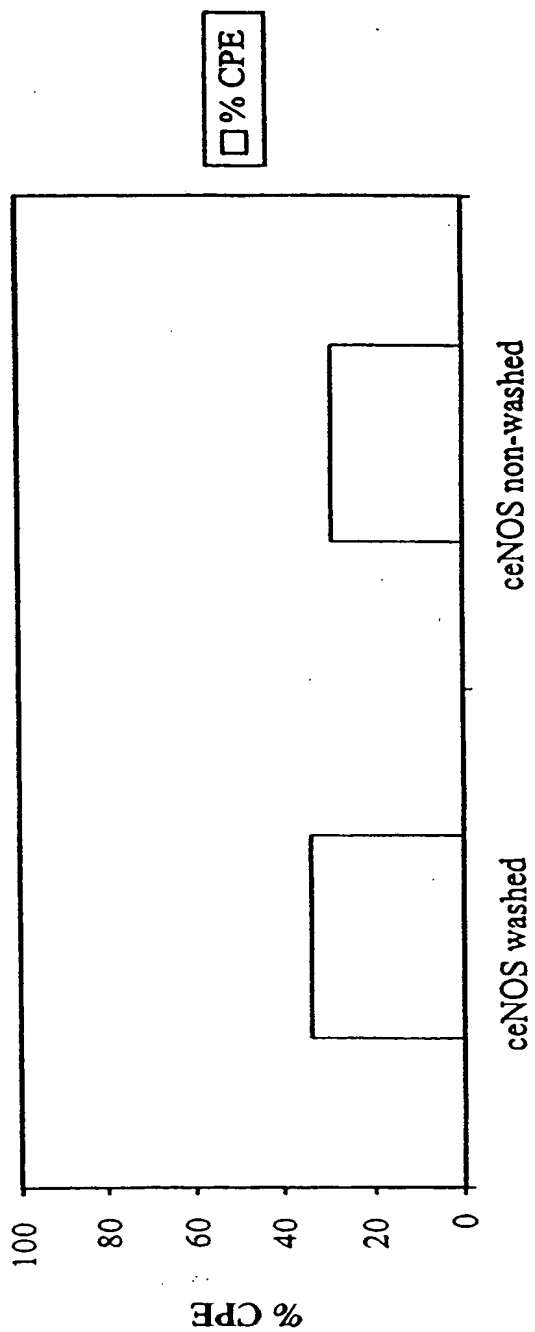


FIG. 44